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10/677,956

Attorney Docket No. 323-100US-D



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In re Application of:	)	Group Art Unit: 1648
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ZEBEDEE et al.	)	Examining Attorney:
	)	Zachariah Lucas
Serial No.: 10/677,956	)	
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For: METHODS AND SYSTEMS FOR	)	
PRODUCING RECOMBINANT	)	
VIRAL ANTIGENS	)	

### DECLARATION OF JOSEPH E. MUETH

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I, Joseph E. Mueth, declare:

I am counsel of record in the above-identified patent application.

I am a member of The State Bar of California and have been admitted to practice before the United States Court of Appeals for the Federal Circuit, the Supreme Court of the United States, and other State and Federal Courts.

I am admitted to practice before the United States Patent and Trademark Office.

Attached are the file histories of:

(1) Wang United States Patent Application Serial No. 07/481,348, filed February 16, 1990, abandoned.

(2) Wang United States Patent Application Serial No. 07/510,153, filed April 16, 1990, abandoned.

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(3) Wang United States Patent Application Serial No. 07/558,799, filed July 26, 1990, issued as United States Patent No. 5,106,726, April 21, 1992.

The file histories are as provided to the undersigned by the United States Patent and Trademark Office, and are for the convenience of the Examiner.

The Examiner will note that Serial Nos. 07/510,153 and 07/481,348 do not disclose any amino acid sequences of the HCV capsid antigen.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: January 31, 2007

Respectfully submitted,

  
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TITLE SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV,  
DIAGNOSIS OF HVC INFECTION AND PREVENTION THEREOF AS VACCINES



This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application as originally filed which is identified above.

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2 UNITED STATES PATENT APPLICATION

3 of

4 Chang Yi Wang

5 for

6 SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION  
7 OF ANTIBODIES TO HCV, DIAGNOSIS OF HCV INFECTION  
8 AND PREVENTION THEREOF AS VACCINES.

9  
10 INTRODUCTION

11  
12 The present invention relates to peptide compositions  
13 specific for the diagnosis and prevention of hepatitis C virus  
14 (HCV) infection, or non-A non-B hepatitis (NANBH). More  
15 particularly, the present invention is directed to synthetic  
16 peptide compositions which are specific for the detection of  
17 antibodies to HCV in body fluids and immunoassays using the  
18 same. The invention also includes the use of the synthetic  
19 peptide compositions as antigens for eliciting the production  
20 of monoclonal and polyclonal antibodies against HCV and as  
21 immunogens in vaccines for the prevention of NANBH or HCV  
22 infection.

23 In the 1940s, two independent investigators concluded  
24 that there were at least two types of viral hepatitis,  
25 designated as A and B (HAV and HBV) and that infection by one  
26 type, either HAV or HBV, did not confer the patient with  
27 cross-immunity (1-3). It was only in the 1970's with the  
28 introduction of serologic markers for hepatitis A and hepatitis  
29 B that it became possible to identify diseases caused by the  
30

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1 two viruses and to distinguish between these two types of  
2 hepatitis clinically and serologically.

3 Subsequently, in 1974, Prince et al. suggested that  
4 many cases of transfusion hepatitis could not be attributed to  
5 HAV or HBV and were caused by an agent other than these  
6 viruses. They proposed naming the agent, hepatitis C virus  
7 (HCV) (4). The presence of another hepatitis causing agent was  
8 subsequently confirmed by Alter et al., who reported that  
9 although the exclusion of commercial blood donors found to  
10 carry hepatitis B surface antigen (HBsAg) significantly reduced  
11 the frequency of post-transfusion hepatitis (5), 7 to 10  
12 percent of the 5 million Americans who received transfusions  
13 each year still developed hepatitis. In 90% of these  
14 post-transfusion hepatitis cases, a specific virus, unrelated  
15 to HAV, HBV, Epstein-Barr virus, cytomegalovirus or other  
16 viruses which occasionally produce liver diseases, was  
17 implicated as the etiologic agent (5). This infection was  
18 designated as non-A non-B hepatitis (NANBH).

19 Over the years, NANBH has been reported in patients  
20 undergoing hemodialysis, recipients of renal transplants (6),  
21 intravenous drug abusers (7) and patients in institutions for  
22 the mentally retarded (8). Further, nurses caring for patients  
23 with NANBH have also been found to contract this disease.

24 Epidemiologic evidence suggests that there may be  
25 three types of NANBH: the water-borne epidemic type; the blood  
26 or needle associated type; and the sporadically occurring  
27 (community acquired) type. However, the number and precise  
28 nature of the causative agents of NANBH still remains not  
29 entirely clear.  
30

1           The acute phase of NANBH is less severe than that of  
2 hepatitis B, and the disease is rarely fatal. However, more  
3 than a third of the individuals who contract NANBH develop a  
4 chronic form of the disease in which they may remain infectious  
5 indefinitely. This chronic state may lead to cirrhosis of the  
6 liver and eventually to liver cancer.

7           Many methods have been developed in an attempt to  
8 detect the putative NANBH viral antigens and antibodies. These  
9 include agar-gel diffusion, counter immunoelectrophoresis,  
10 immunofluorescence microscopy, immunoelectron microscopy,  
11 radioimmunoassay, and enzyme-linked immunosorbent assay using  
12 crude biologic lysates and antibodies from patients. However,  
13 none of these assays are sufficiently sensitive, specific, and  
14 reproducible for use as a diagnostic test for NANBH. Some of  
15 the reactivities detected were later attributable to the  
16 presence of antibodies to host cytoplasmic antigens or low  
17 levels of a rheumatoid-factor-like substance frequently present  
18 in patients with or without hepatic diseases.

19           In the absence of a definitive test for NANBH, the  
20 diagnosis in the past has been one of exclusion. It was based  
21 on the clinical presence of acute hepatitis and the persistent  
22 absence of serologic markers for hepatitis A and B,  
23 Epstein-Barr virus or cytomegalovirus.

24           Because no specific test for the detection of  
25 antibodies to NANBH or HCV has been available, the use of  
26 nonspecific tests to screen donors has been adopted in the past  
27 decade as a means of preventing at least some post-transfusion  
28 NANBH.

29           One such surrogate test measures liver enzyme levels.  
30 The concentrations of some of the liver enzymes, in particular

1 alanine aminotransferase (ALT), are frequently elevated in the  
2 blood of patients with active hepatitis. Two independent  
3 studies have shown a correlation between donor ALT levels and  
4 the incidence of NANBH in transfusion recipients (9-11).  
5 However, some studies showed that only about 20 percent of  
6 blood donors who transmitted NANBH have elevated liver enzyme  
7 concentrations. Other investigators, furthermore, have found  
8 that the liver enzyme levels can be increased by extraneous  
9 factors, such as heavy drinking.

10 Epidemiologic circumstances predisposing donor  
11 populations to infection with hepatitis B virus may also favor  
12 exposure to NANBH agents. A study conducted by Stevens et al.  
13 (12) evaluated the risk factors in donors for the presence of  
14 antibodies to hepatitis B virus. The results indicated that  
15 units of blood which were positive for antibodies to the  
16 hepatitis B core antigen (anti-HBc) appeared to present a two  
17 to three-fold greater risk of NANBH in the recipients than  
18 units without anti-HBc. They concluded that anti-HBc screening  
19 of donors might prevent about one third of the cases of NANBH  
20 attributable to transfusion, whereas ATL screening might  
21 prevent nearly one half of the cases of post transfusion NANBH.

22 Even with the use of these surrogate tests to  
23 establish the diagnosis of NANBH by exclusion, the correct  
24 identification of the NANBHV carriers was still far from  
25 satisfactory. Firstly, there are a significant number of  
26 patients who received blood lacking the surrogate markers and  
27 yet developed NANBH. Secondly, there is a minimal overlap  
28 between donors with elevated ALT levels and those with  
29 anti-HBc. Lastly, there are recipients of blood units which  
30

1 were positive for a surrogate marker, but who did not become  
2 infected with NANBHIV (or HCV) (13-15).

3 Thus, there is an urgent demand for a sensitive and  
4 specific method to identify carriers of NANBHIV and to screen  
5 out contaminated blood or blood products. In addition, there  
6 is also a need for an effective vaccine and/or therapeutic  
7 agent for the prevention and/or treatment of the disease.

8 Recently, a group of scientists at Chiron Corp.  
9 constructed a random-primed complementary DNA (cDNA) library  
10 from plasma containing the uncharacterized NANBH agent (16).  
11 They screened the library with serum from a patient diagnosed  
12 with NANBH and isolated a cDNA clone that encodes an antigen  
13 associated specifically with NANBH. This clone was found to be  
14 derived from the genome of an agent similar to the togaviridae  
15 or flaviviridae (16). The newly identified NANBH agent was  
16 called hepatitis C virus (HCV). A specific assay for this  
17 blood-borne NANBH virus was developed based on a fusion  
18 polypeptide of human superoxide dismutase (SOD) and 363 HCV  
19 amino acids, designated as SOD/HCV C100-3 (17). SOD/HVC C-100  
20 was synthesized utilizing a clone of recombinant yeast,  
21 purified, and used to capture circulating viral antibodies  
22 (17). A family of cDNA sequences derived from this hepatitis C  
23 virus was subsequently reported in detail (18).

24 Synthetic peptides have been used increasingly to map  
25 antigenic or immunogenic sites on the surface of proteins, an  
26 approach recently termed "site-directed-serology". The present  
27 inventor (Wang, C.) and a colleague have taken this approach to  
28 identify and characterize highly antigenic epitopes on the  
29 envelope proteins of HIV and to develop sensitive and specific  
30 immunoassays for the detection of antibodies to HIV (previously  
designated HTLV-III) (19-21). See also U.S. Patent 4,735,896,

1 issued April 5, 1988 and U.S. Patent 4,879,212 issued Nov. 7,  
2 1989, the contents of which are, hereby, fully incorporated by  
3 reference (22, 23). Subsequently, a series of finely mapped  
4 and well-characterized HTLV-1/II related synthetic peptides  
5 were employed in the development of synthetic peptide-based  
6 diagnostic assays for the detection of HTLV-1/II antibodies in  
7 infected individuals (24, 25). See also U.S. Patent 4,833,071  
8 issued May 23, 1989, U.S.S.N. 07/297,635 filed January 13, 1989  
9 and USSN 07/469,294 filed January 24, 1990. These assays have  
10 provided superior sensitivity, excellent specificity, and, in  
11 certain cases, an unmatched capability to differentiate  
12 infections with two closely related viruses, thus overcoming  
13 many of the existing problems associated with biologically-  
14 derived tests based on either viral lysate or recombinant  
15 DNA-derived protein.

16 It is, therefore, an objective of the present  
17 invention to develop a detection or diagnostic procedure to  
18 identify and monitor HCV infection.

19 Another objective is to develop a test procedure that  
20 is highly sensitive and accurate.

21 A further objective is to chemically synthesize a test  
22 reagent which can then be used to detect the presence of  
23 antibodies to HCV in body fluids and diagnose NANBH.

24 Another objective is to develop a vaccine which, when  
25 introduced into healthy mammals, including humans, will  
26 stimulate production of efficacious antibodies to HCV, thereby  
27 providing protection against HCV infection.

28 A further objective is to provide a synthetic  
29 immunogen which can be used in mammals for the development of  
30 monoclonal and polyclonal antibodies to HCV.

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#### BRIEF DESCRIPTION OF THE INVENTION

According to the present invention, a series of synthetic peptides representing an immunodominant region of a hepatitis C virus (HCV) protein, each arranged in a specific sequence, has been identified and made by solid phase peptide synthesis. These peptides have been found to be useful in a highly sensitive and accurate method for the detection of antibodies to HCV in sera and body fluids and the diagnosis of non-A non-B hepatitis (NANBH). Because of their high immunoreactivity, it is expected that these peptides are also useful in stimulating production of antibodies to HCV in healthy mammals such as Balb/C mice, and in a vaccine composition to prevent HCV or NANBIV infection.

According to the present invention, a peptide composition useful for the detection of antibodies to HCV and diagnosis of NANBH comprises a peptide selected from the group of peptides with the following sequences:

- (i) EE,CSQHL, PYIEQ,GMMLA, EQFKO, KALGL, LOTAS, RQAEV, IAP-X (I)
  - (ii) II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKO, KALGL-X (II)
  - (iii) CV, VIVGR, VVLGG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYI-X (III)
- wherein X is -OH or -NH<sub>2</sub>, and analogues, segments, mixtures, combinations, conjugates and polymers thereof.

The amino acids in this application are abbreviated as shown herein below:

1 A= Ala- alanine.  
 2 R= Arg- arginine.  
 3 D= Asp- asparagine.  
 4 N= Asn- asparagine.  
 5 Q= Gln- glutamine.  
 6 E= Glu- glutamic acid.  
 7 L= Leu- leucine.  
 8 K= Lys- lysine.  
 9 H= His- histidine.  
 10 T= Thr- threonine.  
 11 G= Gly- glycine.  
 12 O= Ile- isoleucine.  
 13 F= Phe- phenylalanine.  
 14 S= Ser- serine.  
 15 W= Trp- tryptophan.  
 16 Y= Tyr- tyrosine.  
 17 V= Val- valine.  
 18 C= Cys- cysteine.  
 19 P= Pro- proline

20  
 21 An example of a combination is: CV,VIVGR,VVLSG,KPAII,  
 22 PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL,LQTAS,RQAEV,  
 23 IAP-X wherein X is -OH or -NH<sub>2</sub>. An example of a segment of  
 24 Peptide II is: PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,  
 25 KALGL-X wherein X is -OH or -NH<sub>2</sub> (IIF). An example of a  
 26 segment of Peptide III is: SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,  
 27 PYI-X wherein X is -OH or -NH<sub>2</sub> (IIID).

28 The present invention also includes a highly sensitive  
 29 and accurate method of detecting antibodies to HCV in body  
 30 fluids and of diagnosing NANBH comprises the following steps:

1 A. Preparing a peptide composition comprising a  
2 peptide selected from the group having the following amino acid  
3 sequences:

- 4 (i) EE,CSQHL, PYIEQ,GMMLA,EQFKQ,KALGL,LOTAS,RQAEV,IAP-X (I)  
5 (ii) II,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL-X (II)  
6 (iii) CV,VIVGR,VVLGG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYI-X (III)

7 wherein X is -OH or -NH<sub>2</sub>, and analogues, segments, mixtures,  
8 combinations, conjugates and polymers thereof; and

9 B. Using an effective amount of the peptide  
10 composition as the antigen in an immunoassay procedure.

11 Further, according to the present invention, the  
12 peptides by themselves, or when coupled to a protein or a  
13 polymeric carrier of homo or hetero dimers or higher oligomers  
14 by use of homo or hetero functional multivalent cross linking  
15 reagents, or when directly synthesized and conjugated to a  
16 branching polyvalent lysine resin, can be used to stimulate  
17 production of antibodies to HCV in healthy mammals, including  
18 humans. The method comprises introducing an effective amount  
19 of the peptide composition containing each of the individual  
20 peptides, analogues or segments or a mixture or a combination  
21 thereof, or in a polymeric form, into the body of a healthy  
22 mammal by intraperitoneal or subcutaneous injection.

23 Vaccines containing the peptides according to the  
24 present invention as the key immunogen may also be prepared.  
25 It is expected that such vaccine compositions may be useful to  
26 prevent HCV infection or NANBH.

27 BRIEF DESCRIPTION OF THE DRAWINGS

28  
29 Figs. 1-1, 1-2, 1-3 and 1-4 show the amino acid  
30 sequences of the immunodominant region of a HCV protein and

1 precisely delineates the amino acid residues (underlined to  
2 show --- marginal, --- moderate, and --- strong) that  
3 contribute to the immunoreactivities of these HCV peptides with  
4 four representative HCV antibody positive sera. The  
5 immunoreactivities were measured as absorbance at 492nm by an  
6 EIA procedure.

7 Figs 2-1 and 2-2 are comparisons of the signal to  
8 cutoff ratio between the peptide based HCV-EIA of the present  
9 invention and that of the recombinant SOD/HCV C-100 protein  
10 based HCV-EIA. In Fig. 2-1 a well-characterized HCV antibody  
11 positive control at various serum dilutions was used as the  
12 sample. In Fig. 2-2 a panel of serum specimens derived from  
13 serial bleedings of a single individual spanning a period of  
14 seroconversion to anti-HCV reactivity were used as samples.

15 Figs. 3-1 and 3-2 depict the frequency distribution of  
16 the HCV-EIA, using Peptide IIG, signal to cutoff ratios  
17 obtained with 264 normal serum and 264 normal plasma specimens  
18 from commercial sources. The mean s/c ratios for the negative  
19 (n=250) and screened out positive (i.e. n=14) serum specimens  
20 are 0.034 and 7.202 respectively; and for the negative (n=255)  
21 and positive (n=9) normal plasma specimens the mean s/c ratios  
22 are 0.084 and 7.089 respectively.

23 Fig. 4 is a histogram depicting the immunoreactivities  
24 of Peptide IIG with sera from individuals: (a) positive for  
25 HBsAg, (n=50); (b) positive for antibodies to HBe protein,  
26 (n=39); (c) with elevated ( 100 I.U./L) alanine  
27 aminotransferase (ALT) enzyme activity, (n=174); (d) positive  
28 for antibodies to retroviruses HIV-1 (n=100), HIV-2 (n=10),  
29 HTLV-I/II (n=14); all asymptomatic, (total n=124); (e) with  
30 AIDS, ARC (n=200) or ATL (n=170) disease, (total n=270); and  
(f) with autoimmune disease (n=20).

Fig. 5 provides a comparison between EIA results using the peptides of the present invention and recombinant SOD/HCV C-100 by their respective s/cutoff ratios on a panel of repeatably reactive specimens (n=23) obtained from a random donor population.

Figure 6 provides a comparison between a passive hemagglutination assay (PHA) using Peptide IIG of the present invention and the recombinant SOD/HCV C-100 EIA by their respective P/C and s/Cutoff ratios for a panel of SOD/HCV C-100 HCV EIA repeatably reactive specimens (n=20) obtained from a random donor population. For results obtained by the PHA, the agglutination pattern is quantitated by a specially designed optical reading instrument (manufactured by Olympus Corporation) where a P/C ratio of larger than 20 is considered negative whereas a P/C ratio of less than 20 is considered positive.

#### DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, three peptides and their segments have been chemically synthesized for the detection of antibodies to HCV in body fluids, the diagnosis of NANBH, and for the vaccination of healthy mammals by stimulating the production of antibodies to HCV. These peptides are arranged in the following sequences:

- (i) EE,CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAP-X (I)
  - (ii) II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X (II)
  - (iii) CV, VIVGR, VVLSG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYI-X (III)
- wherein X is -OH or -NH<sub>2</sub>.

These peptides may comprise combinations or segments, i.e. longer or shorter peptide chains by having more amino



1 acids added to the terminal amino acids, or by amino acids  
2 removed from either terminal end.

3       These peptides may also comprise analogues to  
4 accommodate strain-to-strain variations among different  
5 isolates of HCV. HCV is indicated to have frequent mutations.  
6 Therefore, it is expected that variant strains exist.  
7 Adjustments for conservative substitutions and selection among  
8 the alternatives where non-conservative substitutions are  
9 involved, may be made in the prescribed sequences. It is  
10 expected that as long as the peptide's immunoreactivity  
11 recognizable by the antibodies to HCV is preserved, analogues  
12 of the synthetic peptide may also comprise substitutions,  
13 insertions and/or deletions of the recited amino acids of the  
14 above sequence.

15       These peptides may also comprise conjugates, i.e.,  
16 they may be coupled to carrier proteins such as bovine serum  
17 albumin (BSA) or human serum albumin (HSA). Furthermore, these  
18 peptides may comprise polymers, i.e., they may be synthesized  
19 on a polymeric resin, such as a branching octameric lysine  
20 resin.

21       The amino acid sequences of the polypeptides useful as  
22 test reagents for the detection of antibodies to HCV in body  
23 fluids and diagnosis of NANBH are selected to correspond to a  
24 partial segment of the amino acid sequence of the HCV protein  
25 designated as HCV C-100.

26       In selecting regions of the HCV protein for epitope  
27 analysis, peptides in the 40mer size range with amino acid  
28 sequences covering the complete HCV C-100 protein were  
29 synthesized. These were tested for their immunoreactivity  
30 with serum from a patient positively diagnosed with HCV

1 infection. Three overlapping peptides designated as I, II and  
2 III, were identified to have specific immunoreactivity with the  
3 positive HCV serum. The amino acid sequences of peptides I, II  
4 and III are as follows:

5  
6 EE,CSQHL, PYIEQ,GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAP-NH<sub>2</sub> (I)  
7 II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-NH<sub>2</sub> (II)  
8 CV, VIVGR, VVLSG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYI-NH<sub>2</sub> (III)  
9

10 The three peptides (I), (II) and (III) span a region  
11 of 70 amino acids: CV, VIVGR, VVLSG, KPAIL, PDREV, LYREF,  
12 DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAP, and were  
13 found to have specific immunoreactivity with the positive  
14 control serum. Table 1 shows the amino acid sequence of this  
15 immunodominant region of the HCV protein, and presents the  
16 amino acid sequence of the three chemically synthesized  
17 peptides, designated as I, II and III and segments (A to F)  
18 thereof. Each of these peptides was coated at 5ug/ml. in a 10mM  
19 sodium bicarbonate buffer (pH 9.5) onto polystyrene microwell  
20 plates and tested in a three step 45 minute enzyme immunoassay  
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Table 1  
CHARACTERIZATION OF THE IMMUNODOMINANT REGION OF THE HCV 500-100 FUSION POLYPEPTIDE:

CV, VINGA, VULSG, KPALL, POREV, LYREF, DEMEE, CSOML, PYIEO, COMPLA, EOPKO, KALSL, LOTAS, ROKEV, IAP	RELATIVE IMMUNOREACTIVITY
IA	SL, LOTAS, ROKEV, IAP 3.0
IB	KO, KALSL, LOTAS, ROKEV, IAP 10.3
IC	PLA, EOPKO, KALSL, LOTAS, ROKEV, IAP 23.9
ID	EO, COMPLA, EOPKO, KALSL, LOTAS, ROKEV, IAP 24.6
IE	HL, PYIEO, COMPLA, EOPKO, KALSL, LOTAS, ROKEV, IAP 38.2
IF	EL, CSOML, PYIEO, COMPLA, EOPKO, KALSL, LOTAS, ROKEV, IAP 45.6
IIA	COMPLA, EOPKO, KALSL 3.1
IIB	PYIEO, COMPLA, EOPKO, KALSL 24.3
IIC	CSOML, PYIEO, COMPLA, EOPKO, KALSL 41.7
IID	DEMEE, CSOML, PYIEO, COMPLA, EOPKO, KALSL 44.9
IIE	LYREF, DEMEE, CSOML, PYIEO, COMPLA, EOPKO, KALSL 57
IIF	POREV, LYREF, DEMEE, CSOML, PYIEO, COMPLA, EOPKO, KALSL 99
IIG	EL, POREV, LYREF, DEMEE, CSOML, PYIEO, COMPLA, EOPKO, KALSL 93.2
IIA	EL, DEMEE, CSOML, PYI 4.9
IIB	EL, LYREF, DEMEE, CSOML, PYI 25.3
IIC	EL, POREV, LYREF, DEMEE, CSOML, PYI 85
IID	SL, KPALL, POREV, LYREF, DEMEE, CSOML, PYI 100
IIE	GR, VULSG, KPALL, POREV, LYREF, DEMEE, CSOML, PYI 99
IIF	CV, VINGA, VULSG, KPALL, POREV, LYREF, DEMEE, CSOML, PYI 95

The underlined amino acid residues are: + marginal, - moderate, or - strong immunoreactivity

1 procedure, described hereinbelow, with a panel of HCV antibody  
2 positive sera at various serum dilutions. Calculations using  
3 on the overall EIA absorbance of all positive sera yielded an  
4 array of immunoreactivity indices represented as % relative  
5 immunoreactivity for each of the synthetic HCV peptides. Two  
6 peptides, designated as 11F and 11D, being 40 mer and 30 mer  
7 in size with the following amino acid sequence respectively:

8 PDREV,LYREF,DEEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL (11F)

9 and

10 SG,KPAII,PDREV,LYREF,DEEE,CSQHL,PYI (11D)

11 were found to have the highest immunoreactivity. The relative  
12 (%) immunoreactivity for each of the 19 HCV peptides listed in  
13 Table 1, as a result of this extensive epitope mapping study,  
14 provided a basis for the delineation of several clusters of  
15 amino acid residues (as underlined), each in a prescribed  
16 sequence, that are involved in or relevant to the antigenic  
17 configuration of the HCV peptides.

18 Assays for antibodies to HCV based upon chemically  
19 synthesized peptides show several advantages over assays  
20 utilizing biologic based immunoabsorbents. The peptides can  
21 easily be synthesized in gram quantities by using automated  
22 solid-phase methods, thus providing a reproducible antigen of  
23 high integrity with consistent yields. Isolation of antigens  
24 from biological systems precludes such reproducibility. More  
25 importantly, non-specific reactivities seen in uninfected  
26 individuals are likely due to the heterogeneity of the  
27 preparations used for assay. This is particularly true for  
28 assays using biologic based immunoabsorbents. In these  
29 processes, the host antigens are frequently co-purified with  
30 the desired viral protein(s). Antibodies to these

1 contaminating antigens are frequently found in normal  
2 individuals, thus resulting in false-positive results.

3 The assay of the present invention clearly minimizes  
4 such false-positive reactions as encountered in the other assay  
5 systems and, at the same time, shows a high sensitivity to  
6 truly positive sera by the substantially increased  
7 signal-to-noise ratio. This increased signal-to-noise ratio  
8 likely results from the purity of the immunoabsorbent. The  
9 assay of the present invention is also highly specific, in that  
10 the mean S/C ratios for HCV carriers are about 80-200 times the  
11 mean S/C of those non-infected individuals. See Figs. 3-1 and  
12 3-2.

13 The peptides useful as solid phase immunoabsorbents  
14 for the detection of antibodies to HCV were synthesized by the  
15 "classical" Merrifield method of solid phase peptide synthesis  
16 using side chain protected t-Boc-amino acids to correspond to  
17 the following amino acid sequences:

18 EE,CSQHL, PYIEQ,GMMLA,EQFKQ,KALGL,LOTAS,RQAEV,IAP-X (I)

19 II,PDREV,LYREF,DEMEE,CSQHL, PYIEQ,GMMLA,EQFKQ,KALGL-X (II)

20 CV,VIVGR,VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQHL, PYI-X (III)

21 PDREV,LYREF,DEMEE,CSQHL, PYIEQ,GMMLA,EQFKQ,KALGL-X (IIF)

22 SG,KPAII,PDREV,LYREF,DEMEE,CSQHL, PYI-X (IIID)

23 wherein X is  $-NH_2$ .

24 Other analogues, segments, combinations of these  
25 peptides may be prepared by varying the amino acid sequences  
26 either by adding, subtracting, substituting, or deleting  
27 desired t-Boc-amino acid(s).

28 Following completion of assembly of the desired  
29 blocked peptide on the resin, the peptide-resin is treated with  
30 anhydrous hydrofluoric acid to cleave the peptide from the

1 resin. Functional groups of amino acids which are blocked  
2 during synthesis by benzyl-derived blocking groups are also  
3 cleaved from the peptide simultaneously. The free peptide is  
4 then analyzed and purified by high performance liquid  
5 chromatography (HPLC) and characterized biochemically by amino  
6 acid analysis.

7 Longer peptides with more than about 50 amino acids  
8 may be prepared conveniently using well known recombinant  
9 methods. The known nucleic acids codons for each of the amino  
10 acids in the peptide may be utilized and synthetic genes  
11 encoding such peptides constructed. The synthetic gene may be  
12 inserted into vector constructs by known techniques, cloned and  
13 transfected into host cells, such as E. coli, yeast. The  
14 secreted polypeptide may then be processed and purified  
15 according to known procedures. The peptides synthesized  
16 according to the above described procedure are highly reactive  
17 with antibodies to HCV and can be used as a highly sensitive  
18 and specific immunoadsorbent for the detection of antibodies to  
19 HCV.

20 Figs. 1-1, 1-2, 1-3 and 1-4 show the amino acid  
21 sequences of the immunodominant region of a HCV protein and  
22 precisely delineates the underlined amino acid residues that  
23 contribute (--- marginally, \_\_\_ moderately, or \_\_\_ strongly)  
24 to the immunoreactivities, measured as A492nm by a peptide  
25 based EIA procedure of these HCV peptides with four  
26 representative HCV antibody positive sera.

27 The peptide based EIA procedure is as follows. 100uL  
28 per well of each of the peptides was coated of 5ug/mL in a pH  
29 9.5 sodium bicarbonate buffer (10mM) onto a polystyrene  
30 microwell plate and the microwell plate was incubated as 37°C

1 for about an hour, washed and dried. The test serum samples  
2 were diluted with PBS containing normal goat serum, gelatin and  
3 TWEEN 20. 200ul of the test serum sample solution was added to  
4 each well and allowed to react for 15 mins. at 37°C. The wells  
5 were washed and enzyme labelled antibodies was used to bind the  
6 HCV-antibody-peptide complex and incubated for another 15 min.  
7 A color developer, e.g. orthophenylenediamine (OPD), was then  
8 added. The reaction was stopped after 15 min by the addition  
9 of 50ul 1.0M H<sub>2</sub>SO<sub>4</sub> and the absorbance of the reaction  
10 mixture read at 492nm with an ELISA reader.

11 As demonstrated in Fig. 1-1, serum sample 1 has little  
12 reactivity with Peptide IA and IB, and marginal reactivity with  
13 Peptide IC. However, its reactivity with Peptide ID increases  
14 significantly, followed by a marginal increase with Peptide IE,  
15 and an additional increase with Peptide IF. This indicates  
16 that in the HCV Peptide I series, two clusters of amino acid  
17 residues, namely EQGMM and EECSQ, are contributing to the  
18 antigenic determinant(s) of the HCV Peptide I. Similarly, a  
19 cluster of residues namely EECSQ is contributing to the  
20 immunoreactivity of the HCV Peptide II series; and another  
21 cluster of residues namely SQRPQ is contributing to the  
22 immunoreactivity of HCV Peptide III series. As shown on the  
23 bottom of Fig. 1-1, a total of four spaced clusters of amino  
24 acid residues representing discontinuous epitopes in this  
25 immunodominant region of the HCV protein is identified as  
26 contributing to the specific HCV immunoreactivity with serum  
27 sample 1.

28 Figure 1-2 illustrates an immunoreactivity profile for  
29 serum sample 2 when tested on a total of 19 overlapping  
30 peptides in the HCV Peptide I, II, and III series. There is a

1 clear difference between the immunoreactivity profiles of serum  
2 samples 1 and 2. The immunodominant epitope, as marked by  
3 residues SGKPA and IIPDREF, is located towards the N-terminal  
4 of the region.

5 Figure 1-3 illustrates an immunoreactivity profile for  
6 serum 3 when tested on the same 19 HCV peptide panel. Through  
7 this extensive epitope mapping analysis, serum sample 3 was  
8 found to have a similar immunoreactivity profile to that of  
9 serum sample 2.

10 Figure 1-4 illustrates an immunoreactivity profile for  
11 serum sample 4 which differs significantly from that of sample  
12 2 and 3, while maintaining some similarity to that of sample 1.

13 In summary, epitope mapping analysis conducted with a  
14 series of 19 overlapping peptides covering an immunodominant  
15 region of HCV, which spans a total of 70 amino acid residues as  
16 illustrated in Table 1, reveals a varying degree of  
17 immunoreactivity among different HCV antibody positive samples  
18 and these HCV peptides. Based on overall EIA absorbance  
19 readings obtained with a panel of 8 HCV positive sera with each  
20 of these 19 HCV peptides (Table 2), a relative (%)  
21 immunoreactivity index is established for each of the peptides  
22 as several clusters of amino acid residues are identified as  
23 contributing strongly, as in the case of PDREV; moderately, as  
24 in the case of SGKPA, EVLYREF, CSQHLPYIEQG; and LAEQFKQ, or  
25 marginally, as in the case of KQKAL, to the HCV  
26 immunoreactivity.



### MCV Peptide Analogues

Specimens	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Blank	0.041	0.041	0.041	0.041	0.041	0.041	0.040	0.045	0.040	0.041	0.041	0.044	0.040	0.045	0.040	0.045	0.040	0.043
WAC	0.067	0.050	0.077	0.064	0.135	0.221	0.045	0.044	0.048	0.046	0.048	0.088	0.074	0.064	0.081	0.073	0.071	0.070
WBC	0.067	0.050	0.077	0.064	0.135	0.221	0.045	0.044	0.048	0.046	0.048	0.088	0.074	0.064	0.081	0.073	0.071	0.070
SEC	0.049	0.055	0.330	0.383	0.828	1.175	0.040	0.042	0.220	0.153	0.249	0.385	0.046	0.043	0.072	0.140	0.177	0.122
1	0.066	0.218	0.905	2.581	2.904	3.337	0.075	0.038	3.219	3.232	3.174	3.116	0.287	0.527	3.245	4.357	3.545	3.613
2	0.062	0.080	0.060	0.031	0.171	0.137	0.066	0.103	0.243	0.516	0.812	2.929	0.191	0.316	2.475	2.473	2.473	2.473
3	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
4	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
5	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
6	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
7	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
8	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
9	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
10	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
11	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
12	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
13	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
14	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
15	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
16	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288	0.094	0.284	1.704	1.521	3.119	3.220
17	0.062	0.080	0.060	0.031	0.171	0.137	0.065	0.058	0.121	0.179	0.372	2.288</						

1 Based on the above-mentioned epitope mapping study,  
2 two representative EIAs were configured using either Peptide  
3 IIG alone, or a mixture of two peptides IIF and IIID as the  
4 solid phase antigen.

5 Figs. 2-1 and 2-2 depict the comparison, by signal to  
6 cutoff ratio, between the peptide based HCV-EIA employing  
7 Peptide IIG, at 5 ug/mL coating concentration, and that of  
8 recombinant SOD/HCV C-100 protein based HCV-EIA. In Fig. 2-1,  
9 a well-characterized HCV antibody positive control at various  
10 serum dilutions was used as the sample. In Fig 2-2, a panel of  
11 serum specimens derived from serial bleedings of a single  
12 individual spanning a period of seroconversion to anti-HCV  
13 reactivity was used. Similar dilution titers and equal ability  
14 to identify date of seroconversion, the two parameters  
15 indicative of each assay's sensitivity, are obtained with the  
16 synthetic peptide based EIA according to the present invention  
17 and rDNA HCV C-100 based EIA, except that the peptide based  
18 assay according to the present invention is more sensitive,  
19 conferring a higher signal to cutoff ratio to its positive  
20 specimens.

21 Fig. 3-1 and 3-2 depict the frequency distribution of  
22 the synthetic peptide based HCV-EIA signal to cutoff ratios,  
23 using Peptide IIG at 5ug/mL as the coating concentration,  
24 obtained with 264 normal serum and 264 normal plasma specimens  
25 from commercial sources. The mean s/c ratios for the negative  
26 (n=250) and screened out positive (i.e. n=14) serum specimens  
27 are 0.034 and 7.202 respectively; for the negative (n=255) and  
28 positive (n=9) normal plasma specimens the mean ratios are  
29 0.084 and 7.089 respectively. A sharp contrast between the  
30 screened out positives and all the negatives is obtained with  
the peptide based HCV-EIA of the present invention.

1           Based on the high degree of sensitivity and  
2           specificity of the peptide composition according to the present  
3           invention in their immunoreactivities to antibodies to HCV, it  
4           is believed that the peptide compositions according to the  
5           present invention may also be useful as vaccines to prevent  
6           NANBIII, and as immunogens for the development of both monoclonal  
7           and polyclonal antibodies to HCV in mammals, including humans.  
8           The peptide compositions when coupled to a protein, or  
9           synthesized on a polymeric carrier resin (e.g., an octameric  
10          lysine resin) or when polymerized to homo or hetero dimers or  
11          higher oligomers by cysteine oxidation, induced disulfied cross  
12          linking, or by use of homo or hetero functional multivalent  
13          cross linking reagents, can be introduced to normal subjects to  
14          stimulate production of antibodies to HCV in healthy mammals.

15           The advantages of using the peptides according to the  
16          present invention are many.

17           Since the peptide composition according to the present  
18          invention is not derived biologically from the virus, there is  
19          no danger of exposing the normal subjects who are to be  
20          vaccinated to the disease.

21           The peptides can be chemically synthesized easily.  
22          This means that there is no involvement with the HCV at any  
23          time during the process of making the test reagent or the  
24          vaccine. Another problem which can be minimized by the process  
25          of the present invention is the false positive results caused  
26          by the presence of antigenic materials from host cells  
27          co-purified with the HCV fusion protein. Certain normal  
28          individuals have antibodies to E. Coli or yeast proteins which  
29          are cross reactive with the antigenic materials from host  
30          cells. Sera from these normal individuals may show a positive  
        response in the immunoassays.

1 Further, with appropriate amino acid modifications or  
2 substitutions, it is expected that various peptide analogues  
3 based on the prescribed amino acid sequence can be synthesized  
4 with properties giving rise to lower background readings or  
5 better binding capacity to solid phases useful for HCV antibody  
6 screening assays.

7 Moreover, because the peptide compositions of the  
8 present invention are synthetically prepared, the quality can  
9 be controlled and as a result, reproducibility of the test  
10 results can be assured. Also, since very small amounts of  
11 peptides are required for each test procedure, and because the  
12 expense of preparing the peptides is relatively low, the cost  
13 of screening body fluids for antibodies to HCV, diagnosis of  
14 NANBH infection, or the preparation of a vaccine is relatively  
15 low.

16 The peptides prepared in accordance with the present  
17 invention can be used to detect HCV infection and diagnose  
18 NANBH by using them as the test reagent in an enzyme-linked  
19 immunoadsorbent assay (ELISA), an enzyme immuno-dot assay, an  
20 agglutination based assay, or other well-known immunoassay  
21 devices. The preferred method is ELISA. The ELISA technique  
22 is exemplified in Example 1 and 2, and the agglutination based  
23 assay in Examples 3 and 4. The Examples are used to illustrate  
24 the present invention and are not to be used to limit the scope  
25 of the invention.

26 It is to be noted that in the following methods, 0.25%  
27 by weight of glutaraldehyde may be added to the coating buffer  
28 to facilitate better peptide binding onto the plates or beads.  
29 Further, horseradish peroxidase (HRPO) conjugated mouse  
30 monoclonal anti-human IgG antibody or the HRPO conjugated

1 second antibodies from any other animal species may be used in  
2 place of the HRP-conjugated goat anti-human IgG as the second  
3 antibody tracer.

4 The gelatin used in these processes can include calf  
5 skin gelatin, pig skin gelatin, fish gelatin or any known  
6 available gelatin proteins, or be replaced with albumin  
7 proteins.

#### 8 EXAMPLE 1

9 Measurement of Relative (%) Immunoreactivity for  
10 synthetic peptide covering an immunodominant region of  
11 the HCV protein C-100 by an Enzyme-linked Immunosorbent Assay

12 Wells or 96-well plates were coated at 4°C overnight  
13 (or 1 hour at 37°C), with each of the nineteen peptides: 1A,  
14 1B, 1C, 1D, 1E, 1F, 11A, 11B, 11C, 11D, 11E, 11F, 11G, 11H,  
15 11I, 11J, 11K, 11L, 11M, and 11N (see Table 1) prepared as  
16 described at 5 µg/mL at 100 µL per well in 10mM Na HCO<sub>3</sub>  
17 buffer, pH 9.5. The peptide coated wells were then incubated  
18 with 250 µL of 3% by weight of gelatin in PBS at 37°C for 1  
19 hour to block non-specific protein binding sites, followed by  
20 three washes with PBS containing 0.05% by volume of TWEEN 20  
21 and then dried. The test specimens were diluted with PBS  
22 containing 20% by volume normal goat serum, 1% by weight  
23 gelatin and 0.05% by volume TWEEN 20 at dilutions of 1:20  
24 volume to volume, respectively. 200 µL of the diluted  
25 specimens were added to each of the wells and allowed to react  
26 for 15 minutes at 37°.

27 The wells were then washed six times with 0.05% by  
28 volume TWEEN 20 in PBS in order to remove unbound antibodies.  
29 Horseradish peroxidase conjugated goat anti-human IgG was used  
30 as a second antibody tracer to bind with the HCV

1 antibody-peptide antigen complex formed in positive wells. 100  
2 ul of peroxidase labeled goat anti-human IgG at a dilution of  
3 1:1800 in 1% by volume normal goat serum, 0.05% by volume TWEEN  
4 20 in PBS was added to each well and incubated at 37°C for  
5 another 15 minutes.

6 The wells were washed six times with 0.05% by volume  
7 TWEEN 20 in PBS to remove unbound antibody and reacted with  
8 100ul of the substrate mixture containing 0.04% by weight  
9 orthophenylenediamine (OPD) and 0.12% by volume hydrogen  
10 peroxide in sodium citrate buffer, pH 5.0.

11 This substrate mixture was used to detect the  
12 peroxidase label by forming a colored product. Reactions were  
13 stopped by the addition of 100 ul of 1.0M H<sub>2</sub>SO<sub>4</sub> and the  
14 absorbance measured using an ELISA reader at 492nm (i.e.  
15 A<sub>492</sub>). Assays were performed in singlet at one specimen  
16 dilution (1:20) with a panel of eight representative HCV  
17 antibody positive sera, along with the specimen diluent blank,  
18 negative, weak reactive and positive reactive controls all in  
19 duplicate.

20 Results obtained from this study are shown in Table  
21 2. According to the EIA absorbance readings at 492nm (y axis)  
22 and the amino acid sequences for each of the corresponding HCV  
23 peptides (x axis), representative immunoreactivity profiles are  
24 plotted for four of the eight sera as shown in Figures 1-1 to  
25 1-4. Relative (%) immunoreactivity index for each of the 19  
26 peptides is calculated against Peptide IIID, the one with the  
27 highest absorbance reading, based on the total absorbance of  
28 eight sera at 492nm (See Tables 1 and 2). Fig. 1 shows the  
29 amino acid sequences of the immunodominant region according to  
30 data presented in Tables 1 and 2, and precisely delineates the

1 amino acid residues (underlined) that contribute  
2 (--- marginally, moderately, and \_\_\_ strongly) to the  
3 immunoreactivities.

4 In summary, epitope mapping analysis conducted with a  
5 series of 19 overlapping peptides covering an immunodominant  
6 region of HCV, spanning a total of 70 amino acid residues as  
7 illustrated in Table 1, reveals a varying degree of  
8 immunoreactivities between different HCV antibody positive  
9 samples and these HCV peptides. Based on this study, several  
10 discontinuous epitopes are located within this immunodominant  
11 region. Contrary to what is speculated by the conventional  
12 wisdom, it is found preferably to have peptides with longer  
13 amino acid chains, ideally longer than 20, synthesized in order  
14 to optimally present these antigenic determinants to HCV  
15 antibodies.

16 Based on the above-mentioned epitope mapping study,  
17 two representative EIAs using either peptide IIG alone or a  
18 mixture of two peptides, IIF and IID, as the solid phase  
19 antigen were configured for the following efficacy studies as  
20 demonstrated in Examples 2 and 3.

#### 21 EXAMPLE 2

##### 22 Detection of Antibodies to HCV by an 23 Enzyme-Linked Immunosorbent Assay

24 Wells of 96-well plates were coated at 4°C overnight  
25 (or for 1 hour at 37°C with either Peptide IIG alone at a  
26 coating concentration of 0.5ug per well (designated as IIG EIA)  
27 or with a mixture of two peptides IIF and IID (designated as  
28 IIF/IID EIA) in a ratio by weight of IIF:IID=1:1 at 1 ug per  
29 well of the mixture in 100 uL 10mM NaHCO<sub>3</sub> buffer pH 9.5. The  
30 peptide coated wells were then incubated with 250 uL of 3% by

1 weight of gelatin in PBS at 37°C for 1 hour to block  
2 non-specific protein binding sites, followed by three more  
3 washes with PBS containing 0.05% by volume of TWEEN 20 and  
4 dried.

5 The test specimens were diluted with PBS containing  
6 20% by volume normal goat serum, 1% by weight gelatin and 0.05%  
7 by volume TWEEN 20 at dilutions of 1:20 volume to volume,  
8 respectively. 200  $\mu$ L of the diluted specimens were added to  
9 each of the wells and allowed to react for 15 minutes at 37°.

10 The wells were then washed six times with 0.05% by  
11 volume TWEEN 20 in PBS in order to remove unbound antibodies.  
12 Horseradish peroxidase conjugated goat anti-human IgG was used  
13 as a second antibody tracer to bind with the HCV  
14 antibody-peptide antigen complex formed in positive wells. 100  
15  $\mu$ L of peroxidase labeled goat anti-human IgG at a dilution of  
16 1:1800 in 1% by volume normal goat serum, 0.05% by volume TWEEN  
17 20 in PBS was added to each well and incubated at 37°C for  
18 another 15 minutes.

19 The wells were washed six times with 0.05% by volume  
20 TWEEN 20 in PBS to remove unbound antibody and reacted with 100  
21  $\mu$ L of the substrate mixture containing 0.04% by weight  
22 orthophenylenediamine (OPD) and 0.12% by volume hydrogen  
23 peroxide in sodium citrate buffer, pH 5.0. This substrate  
24 mixture was used to detect the peroxidase label by forming a  
25 colored product. Reactions were stopped by the addition of 100  
26  $\mu$ L of 1.0M  $H_2SO_4$  and the absorbance measured using an ELISA  
27 reader at 492nm (i.e.  $A_{492}$ ). Assays were performed in  
28 singlet at one specimen dilution (1:20) with all test  
29 specimens. Each plate run is accompanied by a panel of eight  
30 controls including the specimen diluent blank, negative, weak



1 HCV reactive and strong HCV reactive controls, all in  
2 duplicate. The strong reactive control was adjusted by  
3 diluting a HCV positive serum in the specimen dilution buffer  
4 at 1:300, which gave an absorbance value at 492nm of about 1.5  
5 when performed in this standard 45 minute assay procedure. A  
6 cutoff value is calculated based on the following formula:  
7  $Cutoff = (0.1 \times SRC) + NRC$ . Both the raw absorbance  
8 (designated as signal) and the ratio of signal to cutoff are  
9 recorded for all specimens analyzed.

10 The following groups of specimens were analyzed on  
11 either the HCV peptide based EIA according to the present  
12 invention, with the plates coated either with 5 µg/mL of  
13 peptide IIG or a mixture containing 5µg/mL IIF and 5µg/mL IID:  
14

- 15 (a) A well-characterized HCV antibody positive control  
16 based on serum dilutions; (on both IIG and IIF/IID  
17 EIAs)
- 18 (b) a panel of serum specimens derived from serial  
19 bleedings of a single individual spanning a period of  
20 seroconversion to anti-HCV reactivity; (on both IIG  
21 and IIF/IID plates)
- 22 (c) 264 normal serum and 264 normal plasma specimens from  
23 commercial sources; (on IIG plates only)
- 24 (d) individuals positive for HBsAg, (n=30); (on both IIG  
25 and IIF/IID plates)
- 26 (e) individuals positive for antibodies to HBe protein,  
27 (n=39); (on both IIG and IIF/IID plates)
- 28 (f) individuals with elevated (>100 I.U./L) alanine  
29 aminotransferase (ALT) enzyme activity, (n=174); (on  
30 both IIG and IIF/IID plates)

- 1 (g) individuals positive for antibodies to retroviruses  
2 HIV-1(n=100), HIV-2(n=10), HTLV-1/II(n=14); all  
3 asymptomatic. (total n=124); (on both IIG and IIF/IIID  
4 plates)  
5 (h) individuals with AIDS, ARC(n=200) or ATL (n=170)  
6 disease, (total n=270); (on both IIG and IIF/IIID  
7 plates) and  
8 (i) individuals with autoimmune disease (n=20). (on IIG  
9 plates only)  
10 (j) recombinant SOD/HCV C-100 HCV-EIA repeatedly reactive  
11 specimens obtained from a random donor population,  
12 (n=23). (on both IIG and IIF/IIID plates).  
13

14 Results obtained from groups (a) and (b) are presented  
15 in Figs. 2-1 and 2-2 respectively (data obtained on IIG plates  
16 only), from group (c) in Figs. 3-1 and 3-2; from groups (d) to  
17 (i) in Fig. 4, from group (j) in Table 3 and Figs. 5 and 6.

18 In brief, as shown in Figs. 2-1 and 2-2, a comparison,  
19 by signal to cutoff ratio, between the peptide based HCV-EIA of  
20 the present invention employing peptide IIG and that of  
21 recombinant SOD/HCV C-100 protein based HCV-EIA produced by  
22 Chiron/Ortho. Similar dilution titers and equal ability to  
23 identify date of seroconversion, the two parameters indicative  
24 of each assay's sensitivity, are obtained for both assays.  
25 However, the assay according to the present invention is more  
26 sensitive and confers a higher signal to cutoff ratio to its  
27 positive specimens.  
28  
29  
30

Table 3

SAMPLE ID No.	HCV S/C	rUNA		ALT (IU/L)	Anti-HBC (S/C)	(OTHER POSITIVES	Peptide HCV-EIA S/C
		RPT S/C	RPT S/C				
1	161	5.33	5.56	5.56	36/58	2.10	11
2	280	5.76	5.56	5.56	78/58	0.07	10
3	374	1.98	2.45	2.45	20/58	1.97	0.573
4	517	5.79	5.68	5.68	34/56	2.04	11
5	561	1.74	2.75	2.47	21/56	2.46	0.172
6	675	0.93	1.33	1.54	29/56	1.98	0.135
7	720	5.68	5.68	5.68	57/56	0.08	13
8	773	5.56	5.88	5.88	86/56	2.07	8.625
9	797	3.79	4.35	4.29	74/56	0.38	1.802
10	869	5.66	5.59	5.59	35/56	2.45	9.755
11	873	5.66	5.59	5.59	26/56	2.34	1.189
12	1003	1.63	1.24	1.01	31/56	2.02	0.078
13	1073	5.73	5.59	5.59	17/56	0.12	2.594
14	1099	1.72	1.76	1.94	10/56	1.84	0.083
15	1118	5.59	5.79	5.79	10/56	0.31	10.5
16	1336	0.93	1.38	1.38	18/56	2.15	0.010
17	1501	5.75	5.67	5.67	36/56	1.99	5.349
18	1530	1.27	1.48	1.50	23/56	2.30	0.943
19	1557	0.91	1.29	1.28	20/56	2.20	0.385
20	1652	2.08	2.64	2.72	42/56	1.72	0.135
21	1877	5.59	5.63	5.63	65/56	2.16	4.943
22	1940	1.64	1.47	1.17	29/56	2.35	0.052
23	2017	5.60	5.84	5.84	11/56	0.19	6.786

Col. 1,2,3=Ortho's HCV results in s/c.; Col 5=ALT values over cutoff in IU/L;  
Col. 6=Abbott's Anti-HBC results in s/c where results UNDER 1.00 are POSITIVE  
due the competitive binding principle of this assay.

1 As shown in Figs. 3-1 and 3-2, the frequency  
2 distribution of the HCV-EIA signal to cutoff ratios, using  
3 peptide 11G at 5ug/mL as the coating concentration, that was  
4 obtained with 264 normal serum and 264 normal plasma specimens  
5 for commercial sources suggested a repeatably reactive rate of  
6 5.3% and 3.4% respectively. These percentages are relatively  
7 high compared with those reported in field clinical trials  
8 (usually 0.5-1.0%) using the rDNA HCV C-100 based EIA kit  
9 (Chiron/Ortho). However, in the assay according to the present  
10 invention, the mean s/c ratios for the negative (n=250) and  
11 screened out positive (i.e. n=14) serum specimens are 0.034 and  
12 7.202 respectively; for the negative (n=255) and positive (n=9)  
13 normal plasma specimens the mean ratios are 0.084 and 7.089  
14 respectively. Such a sharp contrast between the screened out  
15 positives and all the negatives probably precludes the  
16 likelihood of a high false positive rate. Since the source of  
17 these normal specimens are derived from commercial plasma  
18 centers where the paid donors usually represent a population  
19 with higher incidence of viral markers than the rigorously  
20 monitored blood banks, a higher repeatably reactive rate is  
21 also considered reasonable. Previous clinical studies  
22 indicated that between 7 to 10 percent of patients receiving  
23 transfusions developed NANBH, where 90% of these  
24 post-transfusion hepatitis cases are caused by the NANBHIV(5).  
25 These reports also provide some support to the interpretation  
26 of the data obtained herein that a high reactivity represents a  
27 true positive result.

28 Results obtained from the screening of a total of 677  
29 well-characterized clinical specimens previously categorized  
30 into six groups, from (d) to (i) using a representative lot of

1 plates coated with Peptide IIG, were plotted on a histogram as  
2 shown in Fig. 4.

3         Fifteen out of fifty (i.e. 30%) HBsAg carriers, 3 out  
4 of 39 (i.e. 8%) HBe antibody positive individuals, 43 out of  
5 174 (i.e. 24.7%) individuals with elevated ALT enzyme activity,  
6 8 out of 124 (6.5%) asymptomatic individuals with retroviral  
7 antibodies, 6 out of 270 (i.e. 2.2%) individuals with  
8 retroviral related disease, and 0 out of 20 (i.e. 0%)  
9 individuals with autoimmune disease were found to be repeatedly  
10 reactive with the peptide HCV EIA of the present invention  
11 using peptide IIG. All these positive specimens were also  
12 found to be positive when tested on peptides IIF/IIID HCV EIA,  
13 although with much higher s/cutoff ratios.

14         A much higher percentage of positive cases was found  
15 with those who have abnormal liver functions (24.7%) or  
16 previous infection(s) with Hepatitis B (30% and 8%) when  
17 compared to those with other infections or diseases (e.g. 6.5%,  
18 2.2% and 0%).

19         Note: Sera from HBsAg carriers were kindly provided by the  
20 Infectious Diseases Laboratory of the American Red  
21 Cross; sera from HBe antibody positive donors were  
22 obtained from Boston Biomedic Inc.; sera from  
23 individuals with elevated ALT levels ( $>100$  I.U./L)  
24 were obtained from both Boston Biomedic Inc. and NABL  
25 laboratory; sera from asymptomatic individuals with  
26 retroviral antibodies (HIV-1 and HTLV-1) were obtained  
27 from New York Blood Center, and those with HIV-2  
28 antibodies were from Guinea Bissau of West Africa,  
29 kindly provided by Dr. O. Varnier of Italy;; sera from  
30 patients with ATL were kindly provided by the Japanese

1 Red Cross; sera from patients with AIDS and ARC, were  
2 kindly provided by Dr. D. Knowles at Columbia  
3 University College of Physicians and Surgeons, and Dr.  
4 F. Siegal at the Long Island Jewish Hospital; sera  
5 from patients with various complications of autoimmune  
6 diseases were kindly provided by Dr. N. Chiorazzi of  
7 the Cornell University Medical School. All sera have  
8 been characterized by additional licensed serologic  
9 markers before inclusion in the current study.

10 Table 2 illustrates results obtained with the peptide  
11 based HCV EIA described in this invention on a panel of 23  
12 recombinant HCV EIA repeatably reactive specimens obtained from  
13 a random donor population. Data on each specimen's ALT level  
14 and HBe antibody reactivity are provided as supplemental  
15 information for indirect confirmation of NANBH status of the  
16 positive donors. As can be seen from the Table, all eight  
17 specimens with indirect confirmation of its NANBH status scored  
18 positive in the peptide based EIA according to the present  
19 invention (on both IIG and IIF/IID plates). In addition, four  
20 specimens that scored high on the peptide based assay also  
21 scored as strong positive by the recombinant HCV EIA, thus  
22 further confirm the HCV positivity of these specimens. The  
23 remaining ten specimens that scored negative by the peptide  
24 based EIA according to the present invention all had a marginal  
25 s/cutoff ratio of between 0.9 to 2.6. Fig. 5 provides a direct  
26 correlation between the peptide based HCV-EIA of the present  
27 invention and the recombinant based HCV EIA by their respective  
28 s/cutoff ratios for this panel. Thus, the peptide based HCV  
29 EIA of the present invention can clearly differentiate the  
30 repeatably reactive specimens previously screened out by the

1 rRNA based HCV EIA into two distinct groups, a positive group  
2 which correlated highly to those with other known NANBH markers  
3 and a negative group which probably represents specimens with  
4 extraneous reactivities unrelated to HCV. In addition to its  
5 use as a screening assay, the peptide based HCV EIA may also  
6 function as a positive confirmatory test for the rRNA based HCV  
7 EIA.

8  
9 Note: This well-characterized serum panel was kindly  
10 provided by Dr. C. Fang of the American Red Cross QC  
11 laboratory.

12  
13 EXAMPLE 3

14 Detection of Antibodies to HCV  
15 By an Agglutination Based Assay

16 The presently claimed HCV peptides, synthesized  
17 according to the Merrifield solid phase method, can be  
18 conjugated to bovine serum albumin (BSA) by a simple  
19 crosslinking method in the presence of a low percentage of  
20 glutaraldehyde solution (0.025%), or with other crosslinking  
21 reagents such as m-maleimidobenzoyl-N-hydroxysuccinimide ester  
22 (MBS) according to a previously published procedure  
23 (Biochemistry, 18:690-697, 1979). For example: to 0.32 mL of  
24 a BSA solution (10 mg/mL in 0.01 M phosphate buffer, pH 7.0) at  
25 room temperature is added 0.013 mL of an MBS solution (0.025  
26 mg/mL in dimethylformamide). The amount of MBS added to the  
27 BSA solution can be varied dependent on the optimal molar ratio  
28 of BSA to MBS determined for a specific conjugate studied. The  
29 mixture is stirred at room temperature for 1 hour, after which  
30 it is centrifuged to remove any precipitated albumin. The

1 clarified mixture is then subjected to gel filtration on  
2 Sephadex G-25 and the protein-containing fractions, as detected  
3 by their absorbance at 280 nm, are pooled and stored frozen at  
4 -70°C until needed.

5 The peptides are dissolved in H<sub>2</sub>O at 10 mg/mL. A  
6 predetermined amount of each peptide solution is added dropwise  
7 to the previously activated BSA-MBS solution and stirred at  
8 room temperature for 3 hours. The final peptide-BSA conjugates  
9 are separated from other free peptides by gel filtration or  
10 extensive dialysis. The ratio of peptide to BSA is determined  
11 by SUS-PAGE according to conventional methods.

12 Using the above mentioned peptide-BSA conjugation  
13 process, conjugated peptide IIG-BSA was absorbed to double  
14 aldehyde fixed human O erythrocytes at pH 4.0. The  
15 peptide-conjugate coated erythrocytes were then treated with  
16 NaBH<sub>4</sub> to prevent non-specific protein binding. The  
17 peptide-conjugate coated erythrocytes were then washed with PBS  
18 and incubated with 5% normal human serum-PBS solution. These  
19 processed cells were then used in an agglutination assay for  
20 the detection of HCV antibodies in both serum and plasma  
21 specimens. The specimens were diluted 1:10 in a sample diluent  
22 buffer and an equal volume of the indicator cells (50 uL) was  
23 mixed with the diluted specimens. The agglutination pattern  
24 was settled within one hour; and the assay results were read by  
25 the naked eye and further quantitated by an optical device  
26 (manufactured by Olympus Corporation) which gave a P/C ratio,  
27 as determined by the absorbance readings of the periphery and  
28 center of the wells. In this experiment, a P/C ratio of 20 was  
29 set as the assay cutoff value, i.e. a positive agglutination  
30 pattern had a ratio of 20 and a negative pattern, 20.



1 A total of 20 rDNA HCV EIA repeatably reactive  
2 specimens were tested for antibodies to HCV in the  
3 above-described HCV passive hemagglutination assay (PHA)  
4 employing Peptide IIG-BSA conjugate as the solid phase. Figure  
5 6 provides a correlation study between the peptide based HCV  
6 PHA and the recombinant based HCV EIA (available from  
7 Chiron/Ortho) by their respective P/C and s/cutoff ratios. All  
8 samples with s/c EIA ratios higher than 3 were found to be  
9 positive with the HCV PHA test. With the exception of one, all  
10 specimens having boarderline s/cutoff ratios (between 0.9 to 2)  
11 scored as negative in this PHA test.

12  
13 EXAMPLE 4

14 Detection of Antibodies to HCV By An  
15 Agglutination Assay Utilizing As the Solid Phase  
16 Immunosorbent Gelatin Particles, Erythrocytes  
Of Different Animal Species, Or Latex Particles  
Coated with a Mixture of JCV Peptides

17 One mL thoroughly washed erythrocytes, gelatin  
18 particles, or polystyrene latex particles are coated with the  
19 HCV poptide mixture, or conjugates thereof at an effective  
20 concentration. The peptide mixture, or conjugates thereof,  
21 coated cells or particles are then incubated with serially  
22 diluted serum samples in the wells of a 96-well U-shaped  
23 microplate or on a slide. After being left at room temperature  
24 for about an hour, or a few minutes in the case of latex  
25 particle based microagglutination, the settled agglutination  
26 pattern on the bottom of each well or on the slide is read; and  
27 the highest dilution showing a positive reaction is recorded.

28 This is a one-step assay which can be used for both  
29 qualitative and quantitative detection of antibodies to HCV in  
30 specimens including sera or biofluids.

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EXAMPLE\_5

A test kit for detecting HCV antibodies using an agglutination assay comprises a compartmented enclosure containing multiple microwell plates and other accessory materials for an agglutination assay including (1) a bottle of HCV peptide coated erythrocytes, gelatin particles or latex polystyrene particles; (2) a negative control; and, (3) an inactivated HCV positive control, and (4) specimen diluent. The procedure described in Examples 3 and 4 is to be followed.

EXAMPLE\_6

An enzyme immunoassay based diagnostic test kit for the detection of HCV antibodies can be constructed. The test kit comprises a compartmented enclosure containing multiple 96-well plates coated prior to use with the HCV peptide or peptide mixtures of the present invention in 100 uL pH 9.5 10mM  $\text{NaHCO}_3$  buffer. The kit further comprises materials for enzyme detection in separate sealed containers consisting of: 1) a negative control; 2) an inactivated HCV positive control; 3) specimen diluent; 4) peroxidase labeled-second antibody to human IgG; and 5) a color change indicator consisting of, for example, orthophenylenediamine (OPD) and hydrogen peroxide in a phosphate citrate buffer. The procedure described in Examples 1 and 2 is to be followed.

In this test kit, 96-well plates, precoated with a peptide or peptide mixture of the present invention, can be replaced by polystyrene beads, or multiple mini-columns filled with controlled pore size glass beads, or nitrocellulose paper

1 strip, precoated with the peptides of the present invention for  
2 use as the solid phase immunosorbent.

3  
4 EXAMPLE 7

5 Immunization with Octameric HCV  
6 Peptides for the Elicitation of Sustaining High  
7 Titers of HCV Antibodies.

8 In addition to the use of synthetic HCV peptides as  
9 immunogens for the generation of sequence-related anti-HCV  
10 antibodies for the ultimate development of an epitope-based  
11 subunit NANBH vaccine, another approach using a limited  
12 sequential propagation of a trifunctional amino acid lysine to  
13 form a core that serves as a low-molecular weight matrix  
14 carrier for peptide immunogens can also be applied. The  
15 trifunctional amino acid, Boc-Lys(Boc), is particularly  
16 suitable since both N- $\alpha$  and N- $\epsilon$  amino groups are available as  
17 reactive ends. Thus, sequential propagation of Boc-Lys(Boc)  
18 will generate  $2^n$  reactive ends. The first level coupling of  
19 Boc-Lys(Boc) will produce two reactive amino ends as a bivalent  
20 carrier. The sequential generations of a second and third step  
21 with Boc-Lys(Boc) will produce carriers containing  
22 four(tetra-valent), and eight (octa-valent) reactive amino ends  
23 to which peptide antigens are attached.

24 The HCV peptides as described in this invention can be  
25 incorporated onto this carrier system as illustrated below for  
26 the development of sustaining high titer HCV antibodies in  
27 mammals, including humans.  
28  
29  
30

1	HCV peptide	
2	HCV peptide	K
3	HCV peptide	K
4	HCV peptide	K
5	HCV peptide	K
6	HCV peptide	K
7	HCV peptide	K
8	HCV peptide	K
9		

10 Octameric HCV peptides of the present invention (Table  
11 1) using the solid phase method of Merrifield are synthesized  
12 by an automated peptide synthesizer, either Applied Biosystems  
13 (ABI) Model 430A, or Biosearch Model 9500.

14 Both acid-labile tert-butyloxycarbonyl (t-Boc) and  
15 acid-stable groups are used for the protection of N-amino acid  
16 and the functional side chains of the amino acids during the  
17 synthesis, respectively. The octameric peptides are  
18 synthesized by coupling onto a synthetic octamer resin.

19 An octamer resin is prepared by coupling di-t-Boc Lys  
20 onto 0.14 mmol/g MBHA (4-Methyl benzhydrylamine) resin.  
21 (Biosearch 9500 is used for this preparation due to its  
22 flexibility in scales). Di-Boc Lys single coupling is followed  
23 by two capping reactions (e.g. 0.3M Acetylimidazole in DMF  
24 dimethylformamide). The substitution level of synthetic  
25 octamer resin is determined by Ninhydrin Test.

26 Duncan Hartly random bred female guinea pigs (two per  
27 immunogen), weighing 400-500 gms, are used as the hosts. For  
28 initial immunizations, an aliquot of 100 ug octameric HCV  
29 peptide in 0.5 mL PBS is mixed with an equal volume of complete  
30 Freund's adjuvant and injected into each animal both

1 subcutaneously and intradermally over multiple sites. After  
2 two to three weeks of rest, an identical dosage of the same  
3 imminogen is given as a boost into each animal except that  
4 incomplete Freund's adjuvant is used. The animals are bled by  
5 heart puncture periodically to monitor each serum's anti-HCV  
6 titers. Subsequent booster shots are given periodically.

7 It is to be understood that the above examples are  
8 illustrative of the present invention and are not meant to  
9 limit the scope thereof.

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WE CLAIM:

1. A peptide composition comprising a peptide with an amino acid sequence selected from the group consisting of:

- (i) EE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAP-X (I)
- (ii) II, PUREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X (II)
- (iii) CV, VIVGR, VVLGG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYI-X

wherein X is -OH or -NH<sub>2</sub>; and

- (iv) analogues, segments, mixtures, combinations, conjugates and polymers thereof.

2. A peptide composition according to Claim 1 comprising a combination of Peptides I, II and III and having the amino acid sequence:

CV, VIVGR, VVLGG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAP-X

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

3. A peptide composition according to Claim 1 comprising a segment of Peptide II and having an amino acid sequence selected from the group consisting of:

- (i) CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;
- (ii) DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;
- (iii) LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;
- (iv) PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

1 4. A peptide composition according to Claim 3 and  
2 having an amino acid sequence as follows:

3 PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EOFKO,KALGL-X

4 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
5

6 5. A peptide composition according to Claim 1  
7 comprising a segment of Peptide III and having an amino acid  
8 sequence selected from the group consisting of:

9 (i) II,PDREV,LYREF,DEMEE,CSQHL,PYI-X;

10 (ii) SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYI-X;

11 (iii) GR,VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYI-X;

12 wherein X is - OH or -NH<sub>2</sub> and analogues thereof.  
13

14 6. A peptide composition according to Claim 5 and  
15 having an amino acid sequence as follows:

16 SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYI-X

17 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
18

19 7. A method of detecting, in body fluids, antibodies  
20 to hepatitis C virus (HCV) or diagnosis of HCV infection or  
21 NANBH comprising the steps:

22 (i) Preparing a peptide composition according to  
23 Claim 1;

24 (ii) Using an effective amount of the peptide  
25 composition as an antigen to form a complex  
26 with antibodies to HCV or NANBH;

27 (iii) Detecting the presence of the complex of  
28 peptide with antibodies to HCV or NANBH by an  
29 enzyme linked immunosorbent assay, an  
30

1 immunoradiometric assay or an agglutination  
2 assay or other immunoassays.  
3

4 8. A method according to Claim 7 where in the  
5 peptide composition is coated on a solid substrate.  
6

7 9. A method according to Claim 8 wherein the step  
8 of detecting the presence of the complex of peptide with  
9 antibodies to HCV or NANBHIV is by means of an enzyme linked  
10 immunosorbent assay.  
11

12 10. A method according to Claim 7 wherein the  
13 method of detecting the presence of the complex of peptide with  
14 antibodies to HCV or NANBHIV is by using an immunoradiometric-  
15 assay.  
16

17 11. A method according to Claim 7 wherein the  
18 method of detecting the presence of the complex of peptide with  
19 antibodies to HCV or NANBHIV is by an agglutination assay.  
20

21 12. A method according to Claim 7 wherein the  
22 peptide composition is a combination of peptides I, II and III.  
23 and having the amino acid sequence:

24 CV,VIVGR,VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,  
25 GMMLA,EQFKQ,KALGL,LQTAS,RQAEV,IAP-X

26 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
27

28 13. A method according to claim 7 wherein the  
29 peptide composition comprises a segment of Peptide II and has  
30 an amino acid sequence selected from the group consisting of:



- 1 (i) CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
2 (ii) DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
3 (iii) LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
4 (iv) PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
5 KALGL-X;

6 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.  
7

8 14. A method according to claim 7, wherein the  
9 peptide composition comprises a peptide having an amino acid  
10 sequence:

11 PDREV, LYREF, DEMEE, CSQML, PYIEQ, GMMLA, EQFKQ, KALGL-X  
12 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
13

14 15. A method according to claim 7 wherein the peptide  
15 composition is a segment of peptide III and having an amino  
16 acid sequence selected from the group consisting of:

- 17 (i) II, PDREV, LYREF, DEMEE, CSQKL, PYI-X;  
18 (ii) SG, KPAIL, PDREV, LYREF, DEMEE, CSQKL, PYI-X;  
19 (iii) GR, VVLSG, KPAIL, PDREV, LYREF, DEMEE, CSQKL, PYI-X;

20 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.  
21

22 16. A method according to claim 7 wherein the peptide  
23 composition comprises a peptide having an amino acid sequence:

24 SG, KPAIL, PDREV, LYREF, DEMEE, CSQKL, CSQKL, PYI-X

25 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
26

27 17. Antibodies to HCV or NANBHV produced by using as  
28 an immunogen a peptide composition comprising a peptide with an  
29 amino acid sequence selected from the group consisting of:  
30

1 (i) EE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL, LQTAS,  
 2 RQAEV, IAP-X (I)  
 3 (ii) II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA,  
 4 EQFKQ, KALGL-X (II)  
 5 (iii) CV, VIVGR, VVLSG, KPAIL, PDREV, LYREF, DEMEE,  
 6 CSQHL, PYI-X (EX)  
 7 wherein X is -OH or -NH<sub>2</sub>; and *ILC/W/L/g*  
 8 (iv) analogues, segments, mixtures, combinations,  
 9 conjugates and polymers thereof.

10  
 11 18. Antibodies to HCV or NANBHV according to claim 17  
 12 wherein the immunogen comprises a combination of Peptides I, II  
 13 and III and having the amino acid sequence:

14 CV, VIVGR, VVLSG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYIEQ,  
 15 GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAP-X

16 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

17  
 18 19. Antibodies to HCV or NANBHV according to claim 17  
 19 wherein the immunogen comprises a segment of Peptide II and  
 20 having an amino acid sequence selected from the group  
 21 consisting of:

22 (i) CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
 23 (ii) DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
 24 (iii) LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
 25 (iv) PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
 26 KALGL-X;

27 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

1 20. Antibodies to HCV or NANBHV according to claim 19  
2 wherein the immunogen comprises a peptide composition having an  
3 amino acid sequence as follows:

4 PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL-X  
5 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
6

7 21. Antibodies to HCV or NANBHV according to claim 17  
8 wherein the immunogen comprises a segment of Peptide III and  
9 having an amino acid sequence selected from the group  
10 consisting of:

- 11 (i) II,PDREV,LYREF,DEMEE,CSQKL,PYI-X;  
12 (ii) SG,KPAII,PDREV,LYREF,DEMEE,CSQKL,PYI-X;  
13 (iii) GR,VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQKL,PYI-X;

14 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

15 22. Antibodies to HCV or NANBHV according to claim 21  
16 wherein the immunogen comprises a peptide composition having an  
17 amino acid sequence as follows:

18 SG,KPAII,PDREV,LYREF,DEMEE,CSQKL,PYI-X

19 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
20

21 23. A vaccine composition to prevent HCV infection or  
22 NANBH by using, as an immunogen, a peptide composition  
23 comprising a peptide with an amino acid sequence selected from  
24 the group consisting of:

- 25 (i) EE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL,LQTAS,  
26 RQAEV,IAP-X (I)  
27 (ii) II,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,  
28 EQFKQ,KALGL-X (II)  
29  
30

1 (iii) CV,VIVGR,VVLSG,KPAII,PDREV,LYREF,DEMEE,  
2 CSQHL,PYI-X (III)

3 wherein X is -OH or -NH<sub>2</sub>; and

4 (iv) analogues, segments, mixtures, combinations,  
5 conjugates and polymers thereof.

6  
7 24. A vaccine composition according to claim 23,  
8 wherein the immunogen comprises a combination of Peptides I, II  
9 and III and having the amino acid sequence:

10 CV,VIVGR,VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,  
11 GMMLA,EQFKQ,KALGL,LQTAS,RQAEV,IAP-X

12 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

13  
14 25. A vaccine composition according to claim 23,  
15 wherein the immunogen comprises a segment of Peptide II and  
16 having an amino acid sequence selected from the group  
17 consisting of:

18 (i) CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL-X;

19 (ii) DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KAJGL-X;

20 (iii) LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL-X;

21 (iv) PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,

22 KALGL-X;

23 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

24  
25 26. A vaccine composition according to claim 25,  
26 wherein the immunogen comprises having an amino acid sequence  
27 as follows:

28 PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL-X

29 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

30

1                   27. A vaccine composition according to claim 23,  
2 wherein the immunogen comprises a segment of Peptide III and  
3 having an amino acid sequence selected from the group  
4 consisting of:

- 5                   (i) II, PDREV, LYREF, DEMEE, CSQHL, PYI-X;  
6                   (ii) SG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYI-X;  
7                   (iii) GR, VVLSG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYI-X;  
8 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.  
9

10                   28. A vaccine composition according to claim 27,  
11 wherein the immunogen comprises an amino acid sequence as  
12 follows:

13                   SG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYI-X  
14 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
15

16                   29. An enzyme linked immunosorbent assay (ELISA) test  
17 kit for the detection of antibodies to HCV or NANBHV or the  
18 diagnosis of HCV or NANBHV infection comprising:

- 19                   (i) compartmented enclosure containing multiple  
20                   wells coated with a peptide composition  
21                   according to claim 1;  
22                   (ii) a negative control sample;  
23                   (iii) an inactivated HCV positive control sample;  
24                   (iv) specimen diluent comprising PBS buffer  
25                   containing 20% by volume normal goat serum; 1%  
26                   by weight gelatin and 0.05% by weight TWEEN 20;  
27                   (v) peroxidase labelled antibodies to human IgG; and  
28                   (vi) a color change indicator.  
29  
30

1           30. An ELISA test kit according to claim 29 wherein  
2 the multiple wells are coated with a peptide composition which  
3 is a combination of Peptide I, II and III with an amino acid  
4 sequence:

5           CV,VIVGR,VVLGG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,  
6           GMMLA,EQEKQ,KALGL,LQTAS,RQAEV,IAP-X

7 wherein X is -OH or -NH<sub>2</sub> or analogues thereof.  
8

9           31. An ELISA test kit according to claim 29 wherein  
10 the multiple wells are coated with a peptide composition  
11 comprising a segment of Peptide II and having an amino acid  
12 sequence selected from the group consisting of:

- 13           (i) CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL-X;  
14           (ii) DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL-X;  
15           (iii) LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL-X;  
16           (iv) PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,  
17           KALGL-X;

18 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.  
19

20           32. An ELISA test kit according to claim 29 wherein  
21 the multiple wells are coated with a peptide having an amino  
22 acid sequence:

23           PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL-X

24 wherein X is OH or -NH<sub>2</sub> or an analogue thereof.  
25

26           33. An ELISA test kit according to claim 29 wherein  
27 the multiple wells are coated with a peptide composition  
28 comprising a segment of Peptide III having an amino acid  
29 sequence selected from the group consisting of:  
30

- 1 (i) IL,PDREV,LYREF,DEMEE,CSQHL,PYI-X  
2 (ii) SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYI-X  
3 (iii) GR,VVLSC,KPAII,PDREV,LYREF,DEMEE,CSQHL,PHI-X  
4 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.  
5

6 34. An ELISA test kit according to claim 33 wherein  
7 the Peptide is:  
8 SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYI-X  
9 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
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ABSTRACT

1  
2  
3 The present invention relates to a method for the  
4 detection in body fluids of antibodies to hepatitis C virus  
5 (HCV), also known as a non-A non-B hepatitis (NANBH) virus and  
6 to the diagnosis of NANBH by the use of a composition of  
7 synthetic peptides. Each of these peptides has an amino acid  
8 sequence corresponding to an immunodominant region of a fusion  
9 protein and a polypeptide of HCV, SOD/HCV C100. More  
10 specifically, the present invention is directed to the use of a  
11 group of synthetic peptides in a prescribed sequence or their  
12 analogues for the detection of antibodies to HCV in body  
13 fluids. The detection method includes an enzyme-linked  
14 immunosorbent assay (ELISA), and other forms of immunoassay  
15 procedures.

16 The present invention also relates to a method for  
17 generating high titer antibodies to HCV in healthy mammals,  
18 including humans, by the use of compositions containing these  
19 synthetic peptides, analogues or mixtures thereof, in a free,  
20 conjugated or polymeric form as key components in synthetic  
21 vaccines for the prevention of non-A non-B hepatitis (NANBH).  
22  
23  
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30



PATENT

Docket No. 1151-4028

COMBINED DECLARATION AND POWER OF ATTORNEY FOR  
ORIGINAL DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL,  
DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV,  
DIAGNOSIS OF HCV INFECTION, AND PREVENTION THEREOF AS VACCINES**  
the specification of which

a. ☒ is attached hereto

b. ☐ was filed on \_\_\_\_\_ as application Serial No. \_\_\_\_\_  
and was amended on \_\_\_\_\_ (if applicable).

PCT FILED APPLICATION ENTERING NATIONAL STAGE

c. ☐ was described and claimed in International Application  
No. \_\_\_\_\_ filed on \_\_\_\_\_ and as amended  
on \_\_\_\_\_ (if any).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

☐ I hereby claim foreign priority benefits under Title 35, United States Code § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

☐ The attached 35 U.S.C. § 119 claim for priority for the U.S. application(s) listed below forms a part of this declaration.

Country	Application Number	Date of filing (day, month, yr)	Date of issue (day, month, yr)	Priority Claimed
				<input type="checkbox"/> YES <input type="checkbox"/> NO
				<input type="checkbox"/> YES <input type="checkbox"/> NO
				<input type="checkbox"/> YES <input type="checkbox"/> NO

- Express Mail No.: B108835980 -

PATENT

Docket No. 1151-4028

ADDITIONAL STATEMENTS FOR  
DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) listed below.

Application Serial No.	Filing Date	Status (patented, pending, abandoned)

[ ] In this continuation-in-part application, insofar as the subject matter of any of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorneys and/or agents with full power of substitution and revocation, to prosecute this application, to receive the patent, and to transact all business in the Patent and Trademark Office connected therewith: Jerome G. Lee (Reg. No. 16,967), John D. Foley (Reg. No. 16,836), John A. Diaz (Reg. No. 19,550), Thomas P. Dowling (Reg. No. 19,221), John C. Vassil (Reg. No. 19,098), Warren H. Rotert (Reg. No. 19,659), Alfred P. Ewert (Reg. No. 19,887), David H. Pfeffer, P.C. (Reg. No. 19,825), Harry C. Marcus (Reg. No. 22,390), Robert E. Paulson (Reg. No. 21,046), Stephen R. Smith (Reg. No. 22,615), Kurt E. Richter (Reg. No. 24,052), J. Robert Dailey (Reg. No. 27,434), Eugene Moroz (Reg. No. 25,237), John F. Sweeney (Reg. No. 27,471), Arnold I. Rady (Reg. No. 26,601), Christopher A. Hughes (Reg. No. 26,914), William S. Feiler (Reg. No. 26,720), Joseph A. Calvaruso (Reg. No. 28,287), James V. Gould (Reg. No. 28,859), Richard C. Komson (Reg. No. 27,811), Israel Blum (Reg. No. 26,110) and Joseph A. Bartholomew Verdine (Reg. No. 28,483) of Morgan & Finnegan whose address is: 345 Park Avenue, New York, New York 10154. DeGirolamo (Reg.No.28595)

[ ] I hereby authorize the U.S. attorneys and/or agents named hereinabove to accept and follow instructions from \_\_\_\_\_

as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and/or agents and me. In the event of a change in the person(s) from whom instructions may be taken I will so notify the U.S. attorneys and/or agents named hereinabove.

PATENT

Docket No. 1151-4028

I hereby specify the following as the correspondence address to which all communications about this application are to be directed:

Maria C.H. Lin

SEND CORRESPONDENCE TO:

MORGAN & FINNEGAN, 345 Park Avenue, New York, New York 10154

DIRECT TELEPHONE CALLS TO: (212) 415-8520  
(212) 758-4600

Full name of sole or first inventor Chang Y. Wang

Inventor's signature\* Chang Y. Wang Feb 16, 1990

Residence 159 Hillpark Avenue, Great Neck, New York 11021

Citizenship U.S.A.

Post Office Address United Biomedical Inc., 2 Nevada Drive, Lake Success  
New York 11042

Full name of second joint inventor, if any \_\_\_\_\_

Inventor's signature\* \_\_\_\_\_

date

Residence \_\_\_\_\_

Citizenship \_\_\_\_\_

Post Office Address \_\_\_\_\_

[ ] ATTACHED IS ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY THIRD AND SUBSEQUENT INVENTORS FORM.

\* Before signing this declaration, each person signing must:

1. Review the declaration and verify the correctness of all information therein; and
2. Review the specification and the claims, including any amendments made to the claims.

After the declaration is signed, the specification and claims are not to be altered.

To the inventor(s):

The following are cited in or pertinent to the declaration attached to the accompanying application:

- Express Mail No.: B108 580

Docet No. 1151-4028

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

07/481348

Applicant(s) or Patentee(s): Chang Yi Wang

Serial No. or Patent No.: To be Assigned

Filed or Issued: Herewith

Group Art Unit: To be Assigned

Examiner: To be Assigned

For: SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO  
HCV, DIAGNOSIS OF HCV INFECTION, AND PREVENTION THEREOF IN VACCINES  
VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY  
STATUS (37 CFR 1.9 (f) and 1.27 (c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

☐ the owner of the small business concern identified below:

☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN United Biomedical Inc.

ADDRESS OF CONCERN 2 Nevada Drive  
Lake Success, New York 11042

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concerning the identified above with regard to the invention entitled ANTIBODIES TO SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV, DIAGNOSIS OF HCV INFECTION, AND PREVENTION THEREOF by inventor(s)

AS VACCINES Chang Yi Wang

described in

☒ the specification filed herewith

☐ application Serial No. \_\_\_\_\_, filed \_\_\_\_\_

☐ Patent No. \_\_\_\_\_, issued \_\_\_\_\_

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.29(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Dr. Chang Yi Wang

TITLE OF PERSON OTHER THEN OWNER Chief Executive Officer

ADDRESS OF PERSON SIGNING United Biomedical Inc.

2 Nevada Drive, Lake Success, New York 11042

SIGNATURE Chang Yi Wang

DATE Feb 16, 1990

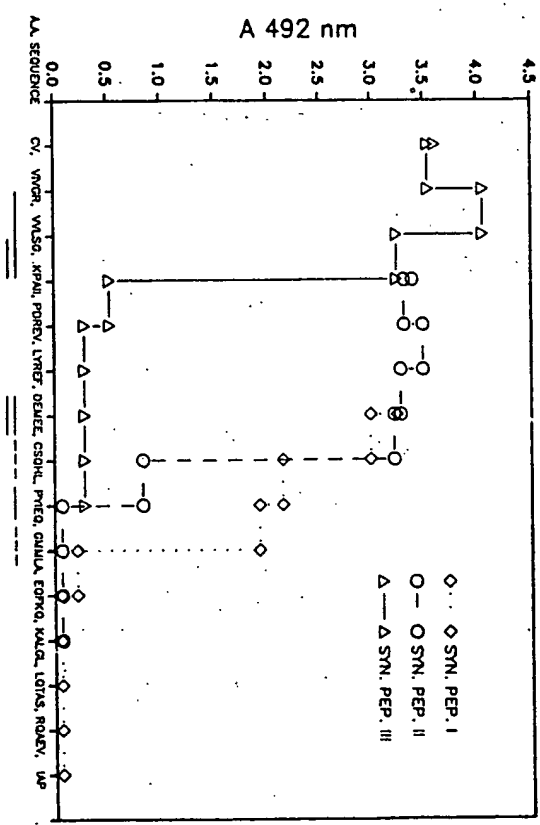
201 2/16 #PCEL

(10950)

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07/481348

EPITOPE MAPPING OF AN IMMUNODOMINANT REGION OF  
THE HCV SOD-C100 FUSION PROTEIN ( sample # 1 )

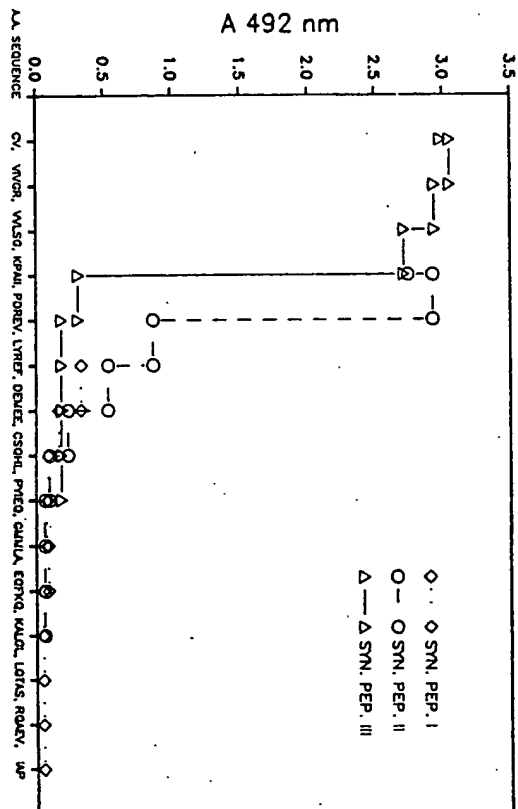


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07/481348

Fig 1-2

EPITOPE MAPPING OF AN IMMUNODOMINANT REGION OF  
THE HCV SOD-C100 FUSION PROTEIN ( sample #2 )

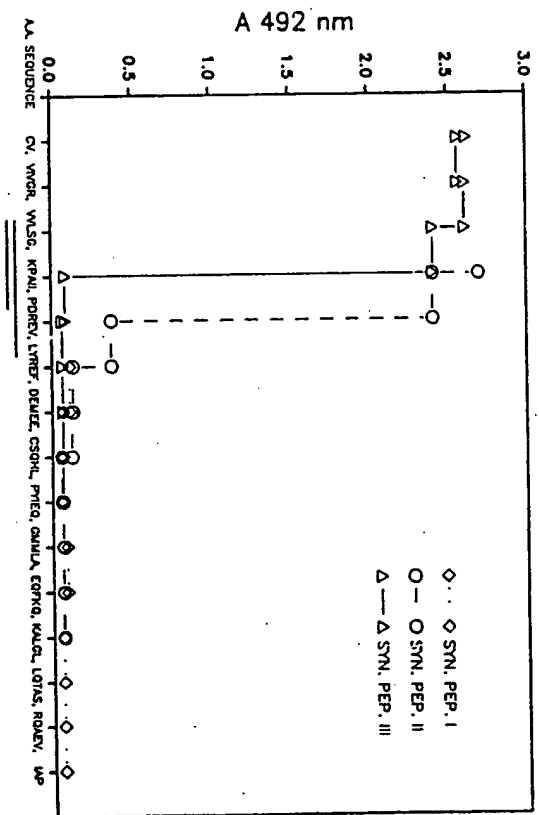


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Fig 1-3

EPITOPE MAPPING OF AN IMMUNODOMINANT REGION OF  
THE HCV SOD-C100 FUSION PROTEIN ( sample #3 )

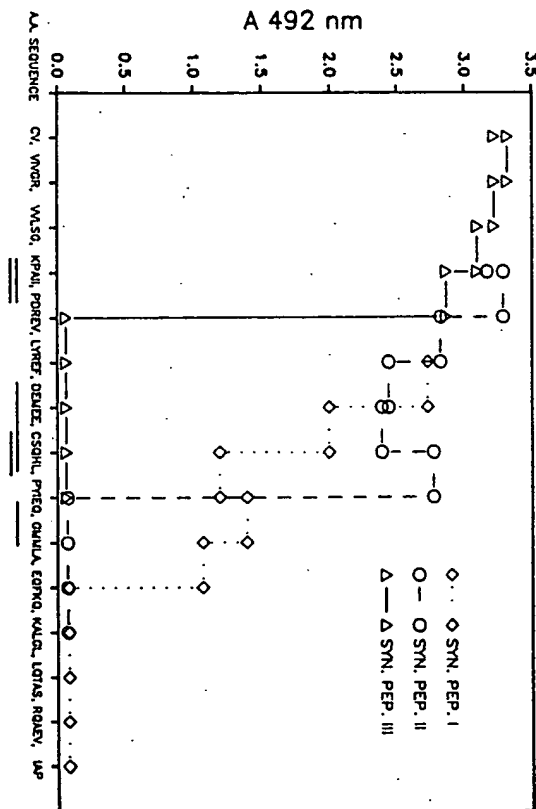


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Fig 1-4

EPITOPE MAPPING OF AN IMMUNODOMINANT REGION OF  
THE HCV SOD-C100 FUSION PROTEIN ( sample #4 )





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Fig. 2-1

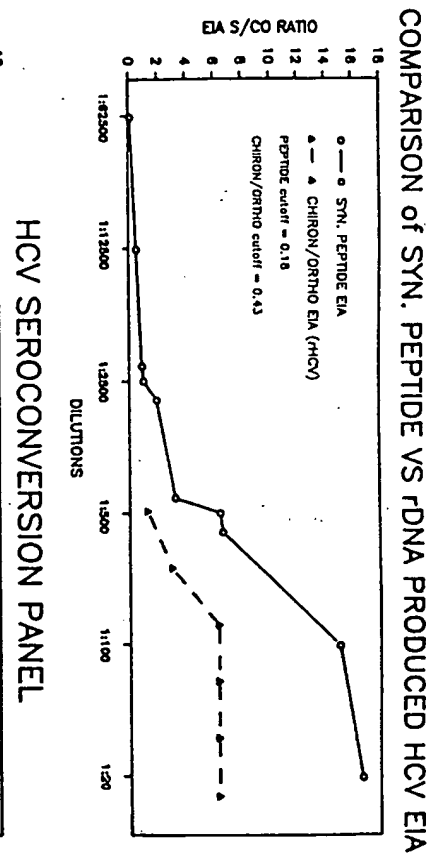
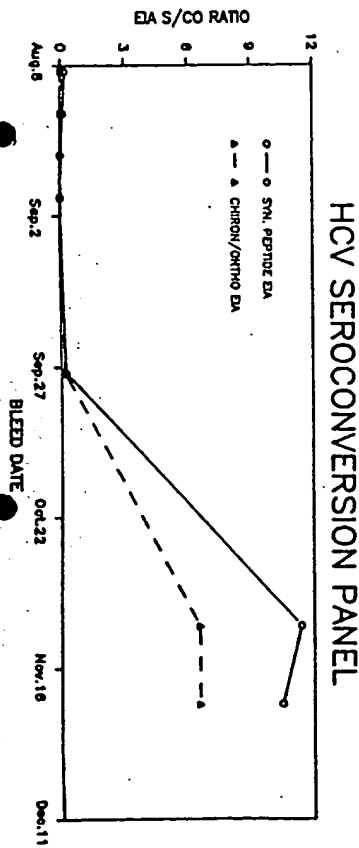


Fig. 2-2



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Fig 3-1

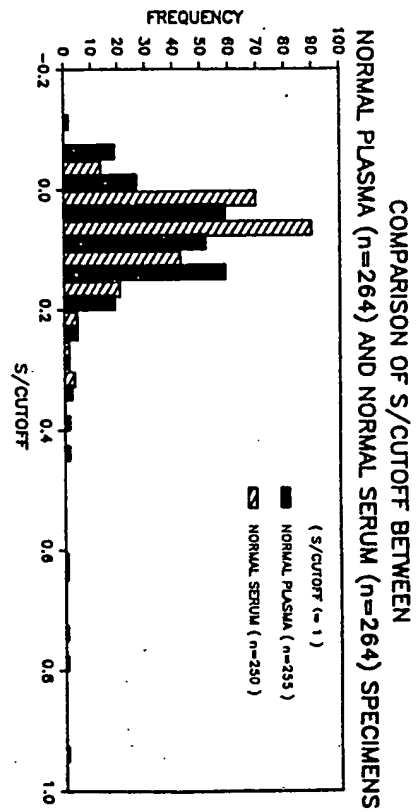
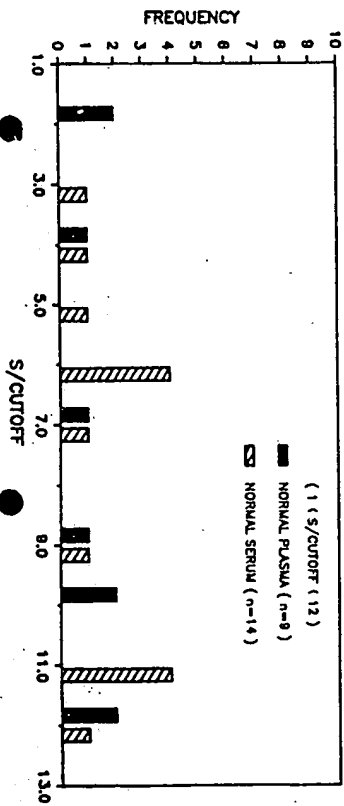


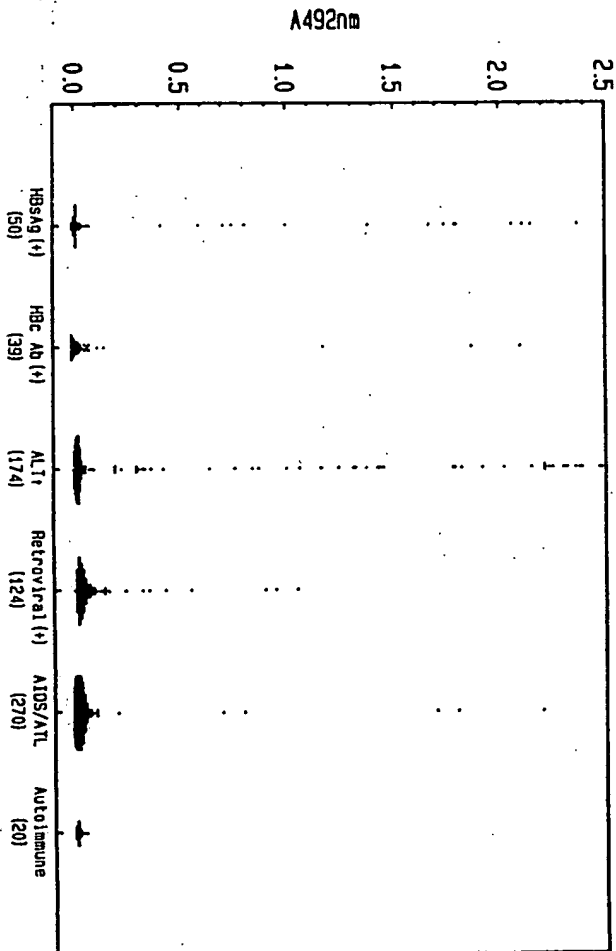
Fig 3-2



H 130391

07/481348

ANTIBODY RESPONSES TO SYNTHETIC HCV PEPTIDE  
IN VARIOUS CLINICAL POPULATIONS



07/481348

Fig 5

COMPARISON of PEPTIDE IIF/IIID and CHIRON/ORTHO HCV EIAs

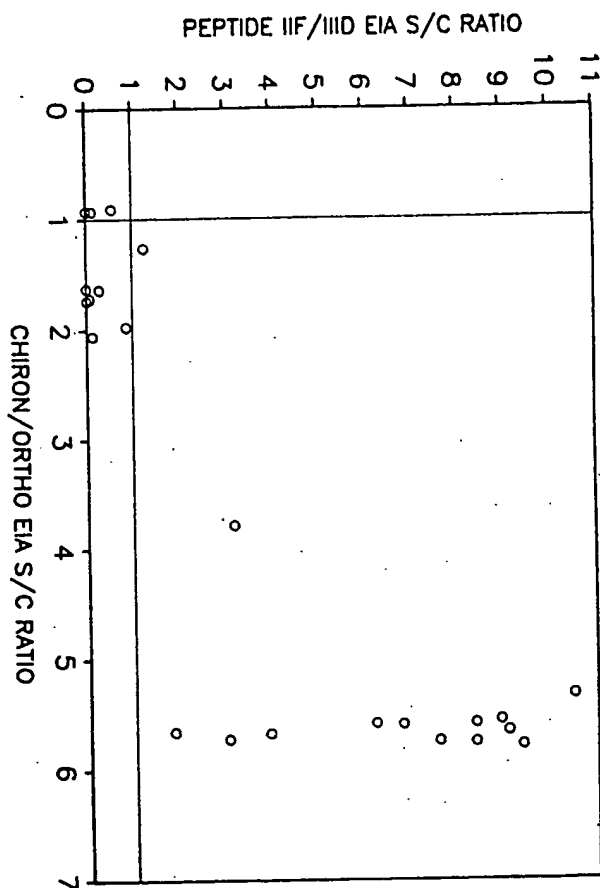
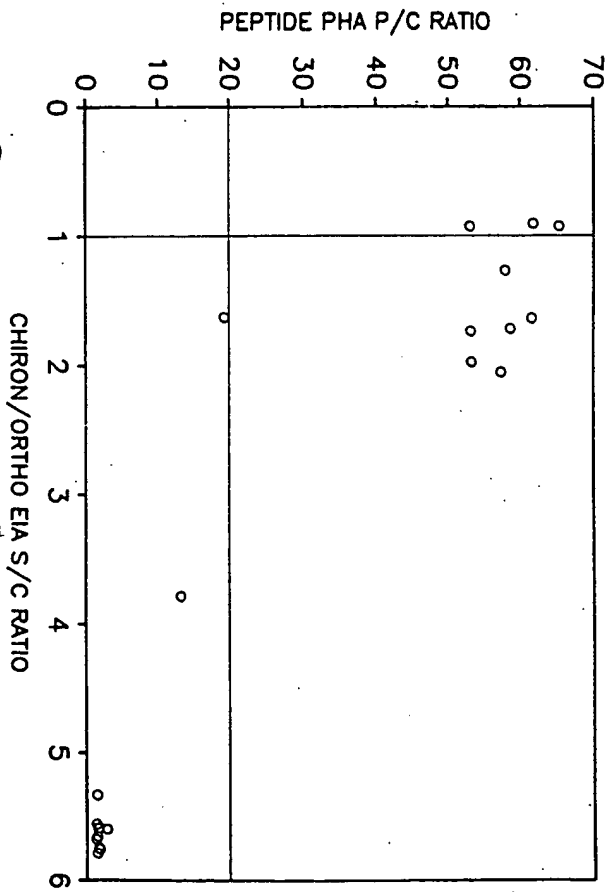


Fig 1

COMPARISON of PEPTIDE HCV PHA and CHIRON/ORTHO HCV EIA



5

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UNITED STATES PATENT APPLICATION

Of

Chang Yi Wang

for

SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION  
OF ANTIBODIES TO HCV, DIAGNOSIS OF HCV INFECTION  
AND PREVENTION THEREOF AS VACCINES.

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation in part application of  
copending application Serial No. 07/481,348, filed February 16,  
1990.

INTRODUCTION

The present invention relates to peptide compositions  
specific for the diagnosis and prevention of hepatitis C virus  
(HCV) infection, or non-A non-B hepatitis (NANBH). More  
particularly, the present invention is directed to synthetic  
peptide compositions which are specific for the detection of  
antibodies to HCV in body fluids and immunoassays using the  
same. The invention also includes the use of the synthetic  
peptide compositions as antigens for eliciting the production  
of monoclonal and polyclonal antibodies against HCV and as  
immunogens in vaccines for the prevention of NANBH or HCV  
infection.

In the 1940s, two independent investigators concluded  
that there were at least two types of viral hepatitis,  
designated as A and B (HAV and HBV) and that infection by one

1 type, either HAV or HBV, did not confer the patient with  
2 cross-immunity (1-2). It was only in the 1970's with the  
3 introduction of serologic markers for hepatitis A and hepatitis  
4 B that it became possible to identify diseases caused by the  
5 two viruses and to distinguish between these two types of  
6 hepatitis clinically and serologically.

7 Subsequently, in 1974, Prince et al. suggested that  
8 many cases of transfusion hepatitis could not be attributed to  
9 HAV or HBV and were caused by an agent other than these  
10 viruses. They proposed naming the agent hepatitis C virus  
11 (HCV) (4). The presence of another hepatitis causing agent was  
12 subsequently confirmed by Alter et al., who reported that  
13 although the exclusion of commercial blood donors found to  
14 carry hepatitis B surface antigen (HBsAg) significantly reduced  
15 the frequency of post-transfusion hepatitis (5), 7 to 10  
16 percent of the 5 million Americans who received transfusions  
17 each year still developed hepatitis. In 90% of these  
18 post-transfusion hepatitis cases, a specific virus, unrelated  
19 to HAV, HBV, Epstein-Barr virus, cytomegalovirus or other  
20 viruses which occasionally produce liver diseases, was  
21 implicated as the etiologic agent (5). This infection was  
22 designated as non-A non-B hepatitis (NANBH).

23 Over the years, NANBH has been reported in patients  
24 undergoing hemodialysis, recipients of renal transplants (6),  
25 intravenous drug abusers (7) and patients in institutions for  
26 the mentally retarded (8). Further, nurses caring for patients  
27 with NANBH have also been found to contract this disease.

28 Epidemiologic evidence suggests that there may be  
29 three types of NANBH: the water-borne epidemic type; the blood  
30 or needle associated type; and the sporadically occurring

1 (community acquired) type. However, the number and precise  
2 nature of the causative agents of NANBH still remain not  
3 entirely clear.

4 The acute phase of NANBH is less severe than that of  
5 hepatitis B, and the disease is rarely fatal. However, more  
6 than a third of the individuals who contract NANBH develop a  
7 chronic form of the disease in which they may remain infectious  
8 indefinitely. This chronic state may lead to cirrhosis of the  
9 liver and eventually to liver cancer.

10 Many methods have been developed in an attempt to  
11 detect the putative NANBH viral antigens and antibodies. These  
12 include agar-gel diffusion, counter immunoelectrophoresis,  
13 immunofluorescence microscopy, immunoelectron microscopy,  
14 radioimmunoassay, and enzyme-linked immunosorbent assay using  
15 crude biologic lysates and antibodies from patients. However,  
16 none of these assays are sufficiently sensitive, specific, and  
17 reproducible for use as a diagnostic test for NANBH. Some of  
18 the reactivities detected were later attributable to the  
19 presence of antibodies to host cytoplasmic antigens or low  
20 levels of a rheumatoid-factor-like substance frequently present  
21 in patients with or without hepatic diseases.

22 In the absence of a definitive test for NANBH, the  
23 diagnosis in the past has been one of exclusion. It was based  
24 on the clinical presence of acute hepatitis and the persistent  
25 absence of serologic markers for hepatitis A and B,  
26 Epstein-Barr virus or cytomegalovirus.

27 Because no specific test for the detection of  
28 antibodies to NANBH or HCV has been available, the use of  
29 nonspecific tests to screen donors has been adopted in the past  
30 decade as a means of preventing at least some post-transfusion  
NANBH.

1           One such surrogate test measures liver enzyme levels.  
2           The concentrations of some of the liver enzymes, in particular  
3           alanine aminotransferase (ALT), are frequently elevated in the  
4           blood of patients with active hepatitis. Two independent  
5           studies have shown a correlation between donor ALT levels and  
6           the incidence of NANBH in transfusion recipients (9-11).  
7           However, some studies showed that only about 20 percent of  
8           blood donors who transmitted NANBH have elevated liver enzyme  
9           concentrations. Other investigators, furthermore, have found  
10          that the liver enzyme levels can be increased by extraneous  
11          factors, such as heavy drinking.

12          Epidemiologic circumstances predisposing donor  
13          populations to infection with hepatitis B virus may also favor  
14          exposure to NANBH agents. A study conducted by Stevens et al.  
15          (12) evaluated the risk factors in donors for the presence of  
16          antibodies to hepatitis B virus. The results indicated that  
17          units of blood which were positive for antibodies to the  
18          hepatitis B core antigen (anti-HBc) appeared to present a two  
19          to three-fold greater risk of NANBH in the recipients than  
20          units without anti-HBc. They concluded that anti-HBc screening  
21          of donors might prevent about one third of the cases of NANBH  
22          attributable to transfusion, whereas ALT screening might  
23          prevent nearly one half of the cases of post transfusion NANBH.

24          Even with the use of these surrogate tests to  
25          establish the diagnosis of NANBH by exclusion, the correct  
26          identification of the NANBHV carriers was still far from  
27          satisfactory. Firstly, there are a significant number of  
28          patients who received blood lacking the surrogate markers and  
29          yet developed NANBH. Secondly, there is a minimal overlap  
30          between donors with elevated ALT levels and those with

1 anti-HBc. Lastly, there are recipients of blood units which  
2 were positive for a surrogate marker, but who did not become  
3 infected with NANBHV (or HCV) (13-15).

4 Thus, there is an urgent demand for a sensitive and  
5 specific method to identify carriers of NANBHV and to screen  
6 out contaminated blood or blood products. In addition, there  
7 is also a need for an effective vaccine and/or therapeutic  
8 agent for the prevention and/or treatment of the disease.

9 Recently, a group of scientists at Chiron Corp.  
10 constructed a random-primed complementary DNA (cDNA) library  
11 from plasma containing the uncharacterized NANBH agent (16).  
12 They screened the library with serum from a patient diagnosed  
13 with NANBH and isolated a cDNA clone that encodes an antigen  
14 associated specifically with NANBH. This clone was found to be  
15 derived from the genome of an agent similar to the togaviridae  
16 or flaviviridae (16). The newly identified NANBH agent was  
17 called hepatitis C virus (HCV). A specific assay for this  
18 blood-borne NANBH virus was developed based on a fusion  
19 polypeptide of human superoxide dismutase (SOD) and 363 HCV  
20 amino acids, designated as SOD/HCV C100-3 (17). SOD/HCV C-100  
21 was produced utilizing a clone of recombinant yeast, purified,  
22 and used to capture circulating viral antibodies (17). A  
23 family of cDNA sequences derived from this hepatitis C virus  
24 was subsequently reported in detail (18).

25 Synthetic peptides have been used increasingly to map  
26 antigenic or immunogenic sites on the surface of proteins, an  
27 approach recently termed "site-directed-serology". The present  
28 inventor (Wang, C.) and a colleague have taken this approach to  
29 identify and characterize highly antigenic epitopes on the  
30 envelope proteins of HIV and to develop sensitive and specific

1 immunoassays for the detection of antibodies to HIV (previously  
2 designated HTLV-III) (19-21). See also U.S. Patent 4,735,896,  
3 issued April 5, 1988 and U.S. Patent 4,879,212 issued Nov. 7,  
4 1989, the contents of which are, hereby, fully incorporated by  
5 reference (22, 23). Subsequently, a series of finely mapped  
6 and well-characterized HTLV-1/II related synthetic peptides  
7 were employed in the development of synthetic peptide-based  
8 diagnostic assays for the detection of HTLV-1/II antibodies in  
9 infected individuals (24, 25). See also U.S. Patent 4,833,071  
10 issued May 23, 1989, U.S.S.N. 07/297,635 filed January 13, 1989  
11 and USSN 07/469,294 filed January 24, 1990. These assays have  
12 provided superior sensitivity, excellent specificity, and, in  
13 certain cases, an unmatched capability to differentiate  
14 infections with two closely related viruses, thus overcoming  
15 many of the existing problems associated with biologically-  
16 derived tests based on either viral lysate or recombinant  
17 DNA-derived protein.

18 It is, therefore, an objective of the present  
19 invention to develop a detection or diagnostic procedure to  
20 identify and monitor HCV infection.

21 Another objective is to develop a test procedure that  
22 is highly sensitive and accurate.

23 A further objective is to chemically synthesize a test  
24 reagent which can then be used to detect the presence of  
25 antibodies to HCV in body fluids and diagnose NANBH.

26 Another objective is to develop a vaccine which, when  
27 introduced into healthy mammals, including humans, will  
28 stimulate production of efficacious antibodies to HCV, thereby  
29 providing protection against HCV infection.  
30

1 A further objective is to provide a synthetic  
2 immunogen which can be used in mammals for the development of  
3 monoclonal and polyclonal antibodies to HCV.  
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10

11 BRIEF DESCRIPTION OF THE INVENTION  
12

13 According to the present invention, a series of  
14 synthetic peptides representing immunodominant regions of a  
15 hepatitis C virus (HCV) protein, each arranged in a specific  
16 sequence, has been identified and made by solid phase peptide  
17 synthesis. These peptides have been found to be useful in a  
18 highly sensitive and accurate method for the detection of  
19 antibodies to HCV in sera and body fluids and the diagnosis of  
20 non-A non-B hepatitis (NANBH). Because of their high  
21 immunoreactivity, it is expected that these peptides are also  
22 useful in stimulating production of antibodies to HCV in  
23 healthy mammals such as Balb/C mice, and in a vaccine  
24 composition to prevent HCV or NANBHV infection.

25 According to the present invention, a peptide  
26 composition useful for the detection of antibodies to HCV and  
27 diagnosis of NANBH comprises a peptide selected from the group  
28 of peptides with the following sequences:  
29  
30

- 1 (i) EE, SCQHL, PYIEQ, GMMLA, EQFKQ, KALGL, LOTAS, RQAEV, IAP-X (I)
- 2 (ii) II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X (II)
- 3 (iii) SG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X (III)
- 4 (iv) CV, VIVGR, VVLSG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYI-X (III)
- 5 (v) EG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQF-X (IV)
- 6 (vi) KQ, KALGL, LOTAS, RQAEV, IAPAV, QTNWQ, KLETF, WAKHM, WNF-X (V)
- 7 (vii) EQ, GMMLA, EQFKQ, KALGL, LOTAS, RQAEV, IAPAV, QTNWQ, KLET-X (VI)
- 8 and
- 9 (viii) PGALV, VGVVC, AAILR, RHVGP, GEGAV, QMMNR, LIAFA, SRGNH, VSP-X (VII)

11 wherein X is -OH or -NH<sub>2</sub>, and analogues, segments, mixtures,  
12 combinations, conjugates and polymers thereof. (The commas in  
13 the above sequences arbitrarily separate the amino acids into  
14 groups of five for convenience.),

15 The amino acids in this application are abbreviated as  
16 shown herein below:  
17

- 18 A= Alg= alanine,
- 19 R= Arg= arginine,
- 20 D= Asp= asparagine,
- 21 N= Asn= asparagine,
- 22 Q= Gln= glutamine,
- 23 E= Glu= glutamic acid,
- 24 L= Leu= leucine,
- 25 K= Lys= lysine,
- 26 H= His= histidine,
- 27 T= Thr= threonine,
- 28 G= Gly= glycine,
- 29 O= Ile= isoleucine,
- 30 F= Phe= phenylalanine,

1 S= Ser= serine.  
 2 W= Trp= tryptophan.  
 3 Y= Tyr= tyrosine.  
 4 V= Val= valine.  
 5 C= Cys= cysteine.  
 6 P= Pro= proline

8 An example of a combination is: CV,VIVGR,VVLGG,KPAII,  
 9 PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL,LQTAS,RQAEV,  
 10 IAP-X wherein X is -OH or -NH<sub>2</sub>. An example of a segment of  
 11 Peptide II is: PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,  
 12 KALGL-X wherein X is -OH or -NH<sub>2</sub> (IIF). An example of a  
 13 segment of Peptide III is: SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,  
 14 PYI-X wherein X is -OH or -NH<sub>2</sub> (IIID).

15 The present invention also includes a highly sensitive  
 16 and accurate method of detecting antibodies to HCV in body  
 17 fluids and of diagnosing NANBH comprises the following steps:

18 A. Preparing a peptide composition comprising a  
 19 peptide selected from the group having the following amino acid  
 20 sequences:

- 22 (i) EE,SCQHL,PYIEQ,GMMLA,EQFKQ,KALGL,LQTAS,RQAEV,IAP-X (I)  
 23 (ii) II,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL-X (II)  
 24 (iii) SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,  
 25 KALGL-X (III)  
 26 (iv) CV,VIVGR,VVLGG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYI-X (III)  
 27 (v) SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQF-X (IV)  
 28 (vi) KQ,KALGL,LQTAS,RQAEV,IAPAV,QTNWQ,KLETG,WAKHM,WNF-X (V)  
 29 (vii) EQ,GMMLA,EQFKQ,KALGL,LQTAS,RQAEV,IAPAV,QTNWQ,KLET-X (VI)  
 30 and  
 (viii) PCALV,VGVVC,AAILR,RHVGP,GEGAV,QMMNR,LIAFA,SRGNH,  
 VSP-X (VII)

1 wherein X is -OH or -NH<sub>2</sub>, and analogues, segments, mixtures,  
2 combinations, conjugates and polymers thereof; and

3 B. Using an effective amount of the peptide  
4 composition as the antigen in an immunoassay procedure.

5 Further, according to the present invention, the  
6 peptides by themselves, or when coupled to a protein or a  
7 polymeric carrier of homo or hetero dimers or higher oligomers  
8 by use of homo or hetero functional multivalent cross linking  
9 reagents, or when directly synthesized and conjugated to a  
10 branching polyvalent lysine resin, can be used to stimulate  
11 production of antibodies to HCV in healthy mammals, including  
12 humans. The method comprises introducing an effective amount  
13 of the peptide composition containing each of the individual  
14 peptides, analogues or segments or a mixture or a combination  
15 thereof, or in a polymeric form, into the body of a healthy  
16 mammal by intraperitoneal or subcutaneous injection.

17 Vaccines containing the peptides according to the  
18 present invention as the key immunogen may also be prepared.

19 It is expected that such vaccine compositions may be useful to  
20 prevent HCV infection or NANBH.

21  
22 BRIEF DESCRIPTION OF THE DRAWINGS

23  
24 Figs. 1-1, 1-2, 1-3 and 1-4 show the amino acid  
25 sequences of the immunodominant region of a HCV protein and  
26 precisely delineates the amino acid residues (underlined to  
27 show --- marginal, \_\_\_ moderate, and \_\_\_\_ strong) that  
28 contribute to the immunoreactivities of these HCV peptides with  
29 four representative HCV antibody positive sera. The  
30 immunoreactivities were measured as absorbance at 492nm by an  
EIA procedure.

1           Figs 2-1 and 2-2 are comparisons of the signal to  
2 cutoff ratio between the peptide based HCV-EIA of the present  
3 invention and that of the recombinant SOD/HCV C-100 protein  
4 based HCV-EIA. In Fig. 2-1 a well-characterized HCV antibody  
5 positive control at various serum dilutions was used as the  
6 sample. In Fig. 2-2 a panel of serum specimens derived from  
7 serial bleedings of a single individual spanning a period of  
8 sero-conversion to anti-HCV reactivity were used as samples.

9           Figs. 3-1 and 3-2 depict the frequency distribution of  
10 the HCV-EIA, using Peptide IIG, signal to cutoff ratios  
11 obtained with 264 normal serum and 264 normal plasma specimens  
12 from commercial sources. The mean s/c ratios for the negative  
13 (n=250) and screened out positive (i.e. n=14) serum specimens  
14 are 0.034 and 7.202 respectively; and for the negative (n=255)  
15 and positive (n=9) normal plasma specimens the mean s/c ratios  
16 are 0.084 and 7.089 respectively.

17           Fig. 4 is a histogram depicting the immunoreactivities  
18 of Peptide IIG with sera from individuals: (a) positive for  
19 HBsAg, (n=50); (b) positive for antibodies to HBc protein,  
20 (n=39); (c) with elevated (>100 I.U./L) alanine  
21 aminotransferase (ALT) enzyme activity, (n=174); (d) positive  
22 for antibodies to retroviruses HIV-1 (n=100), HIV-2 (n=10),  
23 HTLV-I/II (n=14); all asymptomatic, (total n=124); (e) with  
24 AIDS, ARC (N=200) or ATL (n=170) disease, (total n=270); and  
25 (f) with autoimmune disease (n=20).

26           Fig. 5 provides a comparison between EIA results using  
27 the peptides of the present invention and recombinant SOD/HCV  
28 C-100 by their respective s/c ratios on a panel of repeatably  
29 reactive specimens (n=23) obtained from a random donor  
30 population.

1                   Figure 6 provides a comparison between a passive  
2 hemagglutination assay (PHA), using Peptide IIG, and the  
3 recombinant SOD/HCV C-100 EIA by their respective P/C and s/c  
4 ratios for a panel of SOD/HCV C-100 HCV EIA repeatably reactive  
5 specimens (n=20) obtained from a random donor population. For  
6 results obtained by the PHA, the agglutination pattern is  
7 quantitated by a specially designed optical reading instrument  
8 (manufactured by Olympus Corporation) where a P/C ratio of  
9 larger than 20 is considered negative whereas a P/C ratio of  
10 less than 20 is considered positive.

11                   Figure 7-1 provides a study of serum samples collected  
12 over a ten year period of time from a NANBH patient who  
13 sero-converted after receiving HCV infected blood. The samples  
14 were tested by two EIA formats designated as A (coated with  
15 peptides IIF and IIID at 5 ug/mL each) and B (coated with  
16 peptides IIF, IIID and V at 5 ug/mL each) for comparison. The  
17 serum samples were provided by Dr. H. Alter of NIH.

18                   Figure 7-2 provides a kinetic study with serum  
19 samples, kindly provided by Lr. C. Stevens of New York Blood  
20 Center, from a hemodialysis patient who sero-converted and  
21 contracted NANBH. These were tested by EIA format B (coated  
22 with peptides IIF, IIID and V at 5 ug/mL each).

23                   Figure 7-3 provides a second kinetic study with serum  
24 samples, kindly provided by Dr. D. Bradley of Center for  
25 Disease Control, from a chimpanzee which sero-converted after  
26 being inoculated with a well-characterized strain of HCV and  
27 contracted NANBH, also tested by EIA format B.

28                   Figures 8-1 and 8-2 depict the signal/cutoff ratio  
29 frequency distribution of both negative and positive serum  
30 specimens by a HCV-EIA format B. The results were obtained



1 using 2035 low risk random blood donor specimens tested in a  
2 blood bank setting.

3 Figure 9 illustrates the inhibition by peptide IV (an  
4 analogue) of binding of HCV specific antibodies to plates  
5 coated with peptides IID and IIIF at 5 ug/mL each at various  
6 peptide IV concentrations.

7 Figure 10 provides a comparison between the peptide  
8 based HCV EIA (coated with peptide IIH and V at 10 and 5 ug/mL  
9 respectively) and recombinant protein based HCV EIA using  
10 samples from 74 hemodialysis patients, kindly provided by  
11 investigators at the Japanese National Institute of Health.  
12

#### 13 DETAILED DESCRIPTION OF THE INVENTION

14 In accordance with the present invention, three  
15 peptides and their segments have been chemically synthesized  
16 for the detection of antibodies to HCV in body fluids, the  
17 diagnosis of NANBH, and for the vaccination of healthy mammals  
18 by stimulating the production of antibodies to HCV. These  
19 peptides are arranged in the following sequences:  
20

- 21 (i) EE, SQHL, PYIEQ, GMMLA, EQFKQ, KALGI, LQTAS, RQAEV, IAP-X (I)  
22 (ii) II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X (II)  
23 (iii) SG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, (IIH)  
24 KALGL-X  
25 (iv) CV, VIVGR, VVLGG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYI-X (III)  
26 (v) SG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQF-X (IV)  
27 (vi) KQ, KALGL, LQTAS, RQAEV, IAPAV, QTNWQ, KLETF, WAKHM, WNF-X (V)  
28 (vii) EQ, GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAPAV, QTNWQ, KLET-X (VI)  
29 and  
30 (viii) PGALV, VGVVC, AAILR, RHVGP, GEGAV, QMMNR, LIAFA, SRGNH, (VII)  
VSP-X

1 wherein X is -OH or -NH<sub>2</sub>.

2 These peptides may comprise combinations or segments,  
3 i.e. longer or shorter peptide chains by having more amino  
4 acids added to the terminal amino acids, or by amino acids  
5 removed from either terminal end.

6 These peptides may also comprise analogues to  
7 accommodate strain-to-strain variations among different  
8 isolates of HCV. HCV is indicated to have frequent mutations.  
9 Therefore, it is expected that variant strains exist.  
10 Adjustments for conservative substitutions and selection among  
11 the alternatives where non-conservative substitutions are  
12 involved, may be made in the prescribed sequences. It is  
13 expected that as long as the peptide's immunoreactivity  
14 recognizable by the antibodies to HCV is preserved, analogues  
15 of the synthetic peptide may also comprise substitutions,  
16 insertions and/or deletions of the recited amino acids of the  
17 above sequence.

18 These peptides may also comprise conjugates, i.e.,  
19 they may be coupled to carrier proteins such as bovine serum  
20 albumin (BSA) or human serum albumin (HSA). Furthermore, these  
21 peptides may comprise polymers, i.e., they may be synthesized  
22 on a polymeric resin, such as a branching octameric lysine  
23 resin.

24 The amino acid sequences of the polypeptides useful as  
25 test reagents for the detection of antibodies to HCV in body  
26 fluids and diagnosis of NANBH are selected to correspond to a  
27 partial segment of the amino acid sequence of the HCV protein  
28 designated as HCV C-100.

29 In selecting regions of the HCV protein for epitope  
30 analysis, peptides in the 40mer size range with amino acid

1 sequences covering the complete HCV C-100 protein were  
 2 synthesized. These were tested for their immunoreactivity with  
 3 serum from a patient positively diagnosed with HCV infection.  
 4 Six overlapping peptides designated as I, II, III, IV, V and VI  
 5 were identified to have specific immunoreactivity with the  
 6 positive HCV serum. Another peptide VII and its fragments,  
 7 C-terminal to this immunodominant region, was also found to  
 8 have moderate immunoreactivity with a sub population of HCV  
 9 positive sera. See Example 12. Peptide IIH, another analogue  
 10 of Peptide II, with five additional amino acids to the  
 11 N-terminus has been found also to be highly immunogenic. The  
 12 amino acid sequences of the peptides are as follows:

- 13 (i) EE,SCQHL, PYIEQ, GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAP-X (I)
- 14 (ii) II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X (II)
- 15 (iii) SG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X (IIH)
- 16 (iv) CV, VIVGR, VVLSG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYI-X (III)
- 17 (v) SG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQF-X (IV)
- 18 (vi) KQ, KALGL, LQTAS, RQAEV, IAPAV, QTNWQ, KLETF, WAKHM, WNF-X (V)
- 19 (vii) EQ, GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAPAV, QTNWQ, KLET-X (VI)
- 20 and
- 21 (viii) PGALV, VGVVC, AAILR, RHVGF, GEGAV, QMMNR, LIAFA, SRGHI, VSP-X (VII)

22 The six peptides I, II, III, IV, V and VI span a  
 23 region of 90 amino acids: CV, VIVGR, VVLSG, KPAIL, PDREV, LYREF,  
 24 DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAPAV, QTNWQ,  
 25 KLETF, WAKHM, WNF and were found to have specific  
 26 immunoreactivity with the positive control serum. Table 1  
 27 shows the amino acid sequence of this immunodominant region of  
 28 the HCV protein, and presents the amino acid sequence of the

1 six chemically synthesized peptides, designated as I to VI and  
2 segments (A to H) thereof. Each of these peptides was coated  
3 at 5µg/mL in a 10mM sodium bicarbonate buffer (pH 9.5) onto  
4 polystyrene microwell plates and tested in a three step 45  
5 minute enzyme immunoassay  
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Table 1  
CHARACTERIZATION OF THE IMMUNODOMINANT REGION OF THE HCV S20-C100 FUSION POLYPEPTIDE:

	CV, VINGR, VALSG, KPALL, POREV, LYREF, DEMEE, CSQHL, PYIE, GPHLA, EOPKQ, KALGL, LOTAS, ROAEV, LAPAV, QTHMO, KLETF, MAXHH, WHF	RELATIVE (%) IMMUNOREACTIVITY
IA	GL, LOTAS, ROAEV, LAP	3.0
IB	KQ, KALGL, LOTAS, ROAEV, LAP	10.3
IC	MA, EOPKQ, KALGL, LOTAS, ROAEV, LAP	23.9
ID	ED, GPHLA, EOPKQ, KALGL, LOTAS, ROAEV, LAP	38.2
IE	HL, PYIEQ, GPHLA, EOPKQ, KALGL, LOTAS, ROAEV, LAP	45.6
IF	EE, CSQHL, PYIEQ, GPHLA, EOPKQ, KALGL, LOTAS, ROAEV, LAP	3.1
IIA	RYIEQ, GPHLA, EOPKQ, KALGL	24.3
IIB	CSQHL, PYIEQ, GPHLA, EOPKQ, KALGL	41.7
IIC	DEMEE, CSQHL, PYIEQ, GPHLA, EOPKQ, KALGL	44.9
IID	LYREF, DEMEE, CSQHL, PYIEQ, GPHLA, EOPKQ, KALGL	99
IIE	POREV, LYREF, DEMEE, CSQHL, PYIEQ, GPHLA, EOPKQ, KALGL	93.2
IIF	II, POREV, LYREF, DEMEE, CSQHL, PYIEQ, GPHLA, EOPKQ, KALGL	101
IIIA	SG, KPALL, POREV, LYREF, DEMEE, CSQHL, PYIEQ, GPHLA, EOPKQ, KALGL	4.9
IIIB	EF, DEMEE, CSQHL, PYI	26.3
IIIC	CV, LYREF, DEMEE, CSQHL, PYI	85
IID	II, POREV, LYREF, DEMEE, CSQHL, PYI	100
IIE	SG, KPALL, POREV, LYREF, DEMEE, CSQHL, PYI	99
IIF	CV, VINGR, VALSG, KPALL, POREV, LYREF, DEMEE, CSQHL, PYI	95
VIA	AS, ROAEV, LAPAV, QTHMO, KLETF	3.9
VIB	GL, LOTAS, ROAEV, LAPAV, QTHMO, KLETF	43.6
VIC	KQ, KALGL, LOTAS, ROAEV, LAPAV, QTHMO, KLETF	44.0
VIE	LA, EOPKQ, KALGL, LOTAS, ROAEV, LAPAV, QTHMO, KLETF	46.0
VIF	ED, GPHLA, EOPKQ, KALGL, LOTAS, ROAEV, LAPAV, QTHMO, KLETF	54.8
VIA	AV, QTHMO, KLETF, MAXHH, WHF	1.3
VIB	CV, LAPAV, QTHMO, KLETF, MAXHH, WHF	17.8
VIC	II, POREV, LYREF, DEMEE, CSQHL, PYIEQ, GPHLA, EOPKQ, KALGL	23.4
VIE	AS, ROAEV, LAPAV, QTHMO, KLETF, MAXHH, WHF	93.9
VIF	GL, LOTAS, ROAEV, LAPAV, QTHMO, KLETF, MAXHH, WHF	
V	ED, KALGL, LOTAS, ROAEV, LAPAV, QTHMO, KLETF, MAXHH, WHF	

The underlined amino acid residues start ( — ) marginal, ( — ) moderate, or ( — ) strong immunoreactivity

1 procedure, described hereinbelow, with a panel of HCV antibody  
2 positive sera, each selected as representative of a particular  
3 clinical population, at various serum dilutions. Calculations  
4 based on the overall EIA absorbance of all positive sera  
5 yielded an array of immunoreactivity indices represented as %  
6 relative immunoreactivity for each of the synthetic HCV  
7 peptides. Three peptides, designated as IIF, IIH and IIID,  
8 being 40 mer, 47 mer and 30 mer in size with the following  
9 amino acid sequence respectively:

10 PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL (IIF)  
11 SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL (IIH)  
12 and  
13 SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYI (IIID)

14 were found to have the highest immunoreactivity. The relative  
15 (%) immunoreactivity for each of the 30 HCV peptides listed in  
16 Table 1, as a result of this extensive epitope mapping study,  
17 provided a basis for the delineation of several clusters of  
18 amino acid residues (as underlined), each in a prescribed  
19 sequence, that are involved in or relevant to the antigenic  
20 configuration of the HCV peptides.

21 Assays for antibodies to HCV based upon chemically  
22 synthesized peptides show several advantages over assays  
23 utilizing biologic based immunoabsorbents. The peptides can  
24 easily be synthesized in gram quantities by using automated  
25 solid-phase methods, thus providing a reproducible antigen of  
26 high integrity with consistent yields. Isolation of antigens  
27 from biological systems precludes such reproducibility. More  
28 importantly, non-specific reactivities seen in uninfected  
29 individuals are likely due to the heterogeneity of the  
30

1 preparations used for assay. This is particularly true for  
 2 assays using biologic based immunoadsorbents. In these  
 3 processes, the host antigens are frequently co-purified with  
 4 the desired viral protein(s). Antibodies to these  
 5 contaminating antigens are frequently found in normal  
 6 individuals, thus resulting in false-positive results.

7 The assay of the present invention clearly minimizes  
 8 such false-positive reactions as encountered in the other assay  
 9 systems and, at the same time, shows a high sensitivity to  
 10 truly positive sera by the substantially increased  
 11 signal-to-noise ratio. This increased signal-to-noise ratio  
 12 likely results from the purity of the immunoadsorbent. The  
 13 assay of the present invention is also highly specific, in that  
 14 the mean S/C ratios for HCV carriers are about 80-200 times the  
 15 mean S/C of those non-infected individuals. See Figs. 3-1 and  
 16 3-2.

17 The peptides useful as solid phase immunoadsorbents  
 18 for the detection of antibodies to HCV were synthesized by the  
 19 "classical" Merrifield method of solid phase peptide synthesis  
 20 using side chain protected t-boc-amino acids to correspond to  
 21 the following amino acid sequences:

- 22 (i) RE, SCQHL, PYIEQ, GMMLA, EQFKQ, KALGI, LQTAS, RQAEV, IAF-X (I)
- 23 (ii) II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X (II)
- 24 PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X (IIF)
- 25 (iii) SG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X (IIH)
- 26 (iv) CV, VIVGR, VYL SG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYI-X (III)
- 27 SG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYI-X (IIID)
- 28 (v) SG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQF-X (IV)
- 29 (vi) KQ, KALGL, LQTAS, RQAEV, IAPAV, QTNMQ, KLETF, WAKHM, WNF-X (V)
- 30

1 (vii) EQ,GMMLA,EQFKQ,KALGI,LOTAS,RQAEV,IAFAV,QTNMQ,KLET-X (VI)  
2 and

3 (viii) FIALV,VGVVC,AAILR,RHVG,PEGAV,QMMNR,IIAFA,SRGNH, (VII)  
4 VSP-X

5 wherein X is  $-NH_2$ .

6 Other analogues, segments and combinations of these  
7 peptides may be prepared by varying the amino acid sequences  
8 either by adding, subtracting, substituting, or deleting  
9 desired t-Boc-amino acid(s).

10 Following completion of assembly of the desired  
11 blocked peptide on the resin, the peptide-resin is treated with  
12 anhydrous hydrofluoric acid to cleave the peptide from the  
13 resin. Functional groups of amino acids which are blocked  
14 during synthesis by benzyl-derived blocking groups are also  
15 cleaved from the peptide simultaneously. The free peptide is  
16 then analyzed and purified by high performance liquid  
17 chromatography (HPLC) and characterized biochemically by amino  
18 acid analysis.

19 Longer peptides with more than about 50 amino acids  
20 may be prepared conveniently using well known recombinant  
21 methods. The known nucleic acids codons for each of the amino  
22 acids in the peptide may be utilized and synthetic genes  
23 encoding such peptides constructed. The synthetic gene may be  
24 inserted into vector constructs by known techniques, cloned and  
25 transfected into host cells, such as E. coli, or yeast. The  
26 secreted polypeptide may then be processed and purified  
27 according to known procedures. The peptides synthesized  
28 according to the above described procedures are highly reactive  
29 with antibodies to HCV and can be used as a highly sensitive  
30 and specific immunoadsorbent for the detection of antibodies to  
HCV.



1 Figs. 1-1, 1-2, 1-3 and 1-4 show the amino acid  
2 sequences of the immunodominant region of a HCV protein and  
3 precisely delineates the underlined amino acid residues that  
4 contribute (--- marginally, .... moderately, or \_\_\_\_ strongly)  
5 to the immunoreactivities, measured as A492nm by a peptide  
6 based EIA procedure of these HCV peptides with four  
7 representative HCV antibody positive sera.

8 The peptide based EIA procedure is as follows. 100uL  
9 per well of each of the peptides was coated at 5ug/mL in a pH  
10 9.5 sodium bicarbonate buffer (10mM) onto a polystyrene  
11 microwell plate and the microwell plate was incubated at 37°C  
12 for about an hour, washed and dried. The test serum samples  
13 were diluted with PBS containing normal goat serum, gelatin and  
14 TWEEN 20. 200uL of the test serum sample solution was added to  
15 each well and allowed to react for 15 mins. at 37°C. The wells  
16 were washed, enzyme labelled antibodies were used to bind the  
17 HCV-antibody-peptide complex, and the plate was incubated for  
18 another 15 min. A color developer, e.g. orthophenylenediamine  
19 (OPD), was then added. The reaction was stopped after 15 min  
20 by the addition of 50uL 1.0M H<sub>2</sub>SO<sub>4</sub>, and the absorbance of  
21 the reaction mixture was read at 492nm with an ELISA reader.

22 As demonstrated in Fig. 1-1, serum sample 1 has little  
23 reactivity with Peptide IA and IB. However, its reactivity  
24 with Peptide IC increases significantly, followed by a marginal  
25 increase with Peptide ID, and additional increases with  
26 Peptides IE and IF. This indicates that in the HCV Peptide I  
27 series, two clusters of amino acid residues, namely LAEQF and  
28 HLPYI, are contributing to the antigenic determinant(s) of the  
29 HCV Peptide I. Similarly, a cluster of residues namely  
30 EECSQHLPYI is contributing to the immunoreactivity of the HCV

1 Peptide II series; another cluster of residues namely  
2 SGKPAIIPDR is contributing to the immunoreactivity of HCV  
3 Peptide III series and two clusters of residues, namely GLLQT  
4 and EVIAP are contributing to the immunoreactivity by HCV  
5 peptides IV and V series. As shown on the bottom of Fig. 1-1,  
6 a total of six spaced clusters of amino acid residues  
7 representing discontinuous epitopes in this immunodominant  
8 region of the HCV protein are identified as contributing to the  
9 specific HCV immunoreactivity with serum sample 1.

10 Figure 1-2 illustrates an immunoreactivity profile for  
11 serum sample 2 when tested on a total of 31 overlapping  
12 peptides in the HCV Peptide I, II, III, IV, V and VI series.  
13 There is a clear difference between the immunoreactivity  
14 profiles of serum samples 1 and 2. The immunodominant epitope,  
15 as marked by residues SGKPA and IIPNREV, is located towards the  
16 N-terminus of the region.

17 Figure 1-3 illustrates an immunoreactivity profile for  
18 serum 3 when tested on the same 31 HCV peptide panel. Through  
19 this extensive epitope mapping analysis, serum sample 3 was  
20 found to have a similar immunoreactivity profile to that of  
21 serum sample 2.

22 Figure 1-4 illustrates an immunoreactivity profile for  
23 serum sample 4 which differs significantly from that of sample  
24 2 and 3, while maintaining some similarity to that of sample 1.

25 In summary, epitope mapping analysis conducted with a  
26 series of 31 overlapping peptides covering an immunodominant  
27 region of HCV, which spans a total of 90 amino acid residues as  
28 illustrated in Table 1, reveals a varying degree of  
29 immunoreactivity among different HCV antibody positive samples  
30 and these HCV peptides. Based on overall EIA absorbance

1 readings obtained with a panel of 8 HCV positive sera with each  
2 of these 31 HCV peptides (Table 2), a relative (%)  
3 immunoreactivity index is established for each of the peptides  
4 and several clusters of amino acid residues are identified as  
5 contributing strongly, as in the cases of IIPDREVLVR and EVIAP;  
6 moderately, as in the cases of SGKPA, EVLYREF, CSQHLPPYIEQG; and  
7 LAEQFKQ; or marginally, as in the case of KQKAL, to the HCV  
8 immunoreactivity.  
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	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
1	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
2	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
3	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
4	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
5	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
6	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
7	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
8	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
9	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
10	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
11	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
12	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
13	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
14	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
15	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
16	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
17	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
18	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
19	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
20	100	99	95	90	85	80	75	70	65	60	55	50	45	40	35	30	25	20	15	10	5	0
21	100	99	95	90	85	80	75	70	65	60	55											

1 Based on the above-mentioned epitope mapping study,  
2 four representative EIAs were configured using Peptide IIG  
3 alone, a mixture of two peptides IIF and IIID, a mixture of  
4 IIF, IIID and V, or a mixture of IIH and V as the solid phase  
5 antigen.

6 Figs. 2-1 and 2-2 depict the comparison, by signal to  
7 cutoff ratio, between the peptide based HCV-EIA employing  
8 Peptide IIG, at 5 ug/mL coating concentration, and that of  
9 recombinant SOD/HCV C-100 protein based HCV-EIA. In Fig. 2-1,  
10 a well-characterized HCV antibody positive control at various  
11 serum dilutions was used as the sample. In Fig 2-2, a panel of  
12 serum specimens derived from serial bleedings of a single  
13 individual spanning a period of sero-conversion to anti-HCV  
14 reactivity was used. Similar dilution titers and equal ability  
15 to identify date of sero-conversion, the two parameters  
16 indicative of each assay's sensitivity, are obtained with the  
17 synthetic peptide based EIA according to the present invention  
18 and rDNA HCV C-100 based EIA, except that the peptide based  
19 assay according to the present invention is more sensitive,  
20 conferring a higher signal to cutoff ratio to its positive  
21 specimens.

22 Fig. 3-1 and 3-2 depict the frequency distribution of  
23 the synthetic peptide based HCV-EIA signal to cutoff ratios,  
24 using Peptide IIG at 5ug/mL as the coating concentration,  
25 obtained with 264 normal serum and 264 normal plasma specimens  
26 from commercial sources. The mean s/c ratios for the negative  
27 (n=250) and screened out positive (i.e. n=14) serum specimens  
28 are 0.034 and 7.202 respectively; for the negative (n=255) and  
29 positive (n=9) normal plasma specimens the mean ratios are  
30 0.084 and 7.089 respectively. A sharp contrast between the

1 screened out positives and all the negatives is obtained with  
2 the peptide based HCV-EIA of the present invention.

3       Based on the high degree of sensitivity and  
4 specificity of the peptide compositions according to the  
5 present invention in their immunoreactivities to antibodies to  
6 HCV, it is believed that the peptide compositions according to  
7 the present invention may also be useful as vaccines to prevent  
8 NANBH, and as immunogens for the development of both monoclonal  
9 and polyclonal antibodies to HCV in mammals, including humans.  
10 The peptide compositions when coupled to a protein, or  
11 synthesized on a polymeric carrier resin (e.g., an octameric  
12 branching lysine resin) or when polymerized to homo or hetero  
13 dimers or higher oligomers by cysteine oxidation, induced  
14 disulfide cross linking, or by use of homo or hetero functional  
15 multivalent cross linking reagents, can be introduced to normal  
16 subjects to stimulate production of antibodies to HCV in  
17 healthy mammals.

18       The advantages of using the peptides according to the  
19 present invention are many.

20       Since the peptide compositions according to the  
21 present invention are not derived biologically from the virus,  
22 there is no danger of exposing the normal subjects who are to  
23 be vaccinated to the disease.

24       The peptides can be chemically synthesized easily.  
25 This means that there is no involvement with the HCV at any  
26 time during the process of making the test reagent or the  
27 vaccine. Another problem which can be minimized by the process  
28 of the present invention is the false positive results caused  
29 by the presence of antigenic materials from host cells  
30 co-purified with the HCV fusion protein. Certain normal

1 individuals have antibodies to E. Coli or yeast proteins which  
2 are cross reactive with the antigenic materials from host  
3 cells. Sera from these normal individuals may show a positive  
4 response in the immunoassays.

5 Further, with appropriate amino acid modifications or  
6 substitutions, it is expected that various peptide analogues  
7 based on the prescribed amino acid sequence can be synthesized  
8 with properties giving rise to lower background readings or  
9 better binding capacity to solid phases useful for HCV antibody  
10 screening assays.

11 Moreover, because the peptide compositions of the  
12 present invention are synthetically prepared, the quality can  
13 be controlled and as a result, reproducibility of the test  
14 results can be assured. Also, since very small amounts of  
15 peptides are required for each test procedure, and because the  
16 expense of preparing the peptides is relatively low, the cost  
17 of screening body fluids for antibodies to HCV, diagnosis of  
18 NANBH infection, or the preparation of a vaccine is relatively  
19 low.

20 The peptides prepared in accordance with the present  
21 invention can be used to detect HCV infection and diagnose  
22 NANBH by using them as the test reagent in an enzyme-linked  
23 immunoadsorbent assay (ELISA), an enzyme immunodot assay, an  
24 agglutination based assay, or other well-known immunoassay  
25 devices. The preferred method is ELISA. The ELISA technique  
26 is exemplified in Examples 1, 2, 8, 9, 10 and 12 and the  
27 agglutination based assay in Examples 3 and 4. The Examples  
28 are used to illustrate the present invention and are not to be  
29 used to limit the scope of the invention.  
30

1 It is to be noted that in the following methods, 0.25%  
2 by weight of glutaraldehyde may be added to the coating buffer  
3 to facilitate better peptide binding onto the plates or beads.  
4 Further, horseradish peroxidase (HRPO) conjugated mouse  
5 monoclonal anti-human IgG antibody or the HRPO conjugated  
6 second antibodies from any other animal species may be used in  
7 place of the HRPO-conjugated goat anti-human IgG as the second  
8 antibody tracer.

9 The gelatin used in these processes can include calf  
10 skin gelatin, pig skin gelatin, fish gelatin or any known  
11 available gelatin proteins, or be replaced with albumin  
12 proteins.  
13

#### 14 EXAMPLE 1

15 Measurement of Relative (%) Immunoreactivity for  
16 synthetic peptide covering an immunodominant region of  
the HCV protein C-100 by an Enzyme-Linked Immunosorbent Assay

17 Wells or 96-well plates were coated at 4°C overnight  
18 (or 1 hour at 37°C), with each of the twenty peptides: IA, IB,  
19 IC, ID, IE, IF, IIA, IIB, IIC, IID, IIE, IIF, IIG, IIH, IIHA,  
20 IIIB, IIIC, IIID, IIIE, and IIIF (see Table 1) prepared as  
21 described at 5 ug/mL at 100 uL per well in 10mM NaHCO<sub>3</sub>  
22 buffer, pH 9.5. The peptide coated wells were then incubated  
23 with 250 uL of 3% by weight of gelatin in PBS at 37°C for 1  
24 hour to block non-specific protein binding sites, followed by  
25 three washes with PBS containing 0.05% by volume of TWEEN 20  
26 and then dried. The test specimens were diluted with PBS  
27 containing 20% by volume normal goat serum, 1% by weight  
28 gelatin and 0.05% by volume TWEEN 20 at dilutions of 1:20  
29 volume to volume, respectively. 200 uL of the diluted  
30 specimens were added to each of the wells and allowed to react  
for 15 minutes at 37°.



1           The wells were then washed six times with 0.05% by  
2 volume TWEEN 20 in PBS in order to remove unbound antibodies.  
3 Horseradish peroxidase conjugated goat anti-human IgG was used  
4 as a second antibody tracer to bind with the HCV  
5 antibody-peptide antigen complex formed in positive wells. 100  
6  $\mu$ L of peroxidase labeled goat anti-human IgG at a dilution of  
7 1:1800 in 1% by volume normal goat serum, 0.05% by volume TWEEN  
8 20 in PBS was added to each well and incubated at 37°C for  
9 another 15 minutes.

10           The wells were washed six times with 0.05% by volume  
11 TWEEN 20 in PBS to remove unbound antibody and reacted with  
12 100 $\mu$ L of the substrate mixture containing 0.04% by weight  
13 orthophenylenediamine (OPD) and 0.12% by volume hydrogen  
14 peroxide in sodium citrate buffer, pH 5.0.

15           This substrate mixture was used to detect the  
16 peroxidase label by forming a colored product. Reactions were  
17 stopped by the addition of 100  $\mu$ L of 1.0M  $H_2SO_4$  and the  
18 absorbance measured using an ELISA reader at 492nm (i.e.  
19  $A_{492}$ ). Assays were performed in singlet at one specimen  
20 dilution (1:20) with a panel of eight representative HCV  
21 antibody positive sera, along with the specimen diluent blank,  
22 non-reactive, weakly reactive and strongly reactive controls  
23 (NRC, WRC, SRC) all in duplicates.

24           Results obtained from this study are shown in Table  
25  
26 2. According to the EIA absorbance readings at 492nm (y axis)  
27 and the amino acid sequences for each of the corresponding HCV  
28 peptides (x axis), representative immunoreactivity profiles are  
29 plotted for four of the eight sera as shown in Figures 1-1 to  
30 1-4. Relative (%) immunoreactivity index for each of the 31  
peptides is calculated using Peptide IIID as a reference based

1 on the total absorbance of eight sera at 492nm (See Tables 1  
2 and 2). Fig. 1 shows the amino acid sequences of the  
3 immunodominant region according to data presented in Tables 1  
4 and 2, and precisely delineates the amino acid residues  
5 (underlined) that contribute (---marginally, \_\_\_ moderately,  
6 and \_\_\_ strongly) to the immunoreactivities.

7 In summary, epitope mapping analysis conducted with a  
8 series of 31 overlapping peptides covering an immunodominant  
9 region of HCV, spanning a total of 90 amino acid residues as  
10 illustrated in Table 1, reveals a varying degree of  
11 immunoreactivities between different HCV antibody positive  
12 samples and these HCV peptides. Based on this study, several  
13 discontinuous epitopes are located within this immunodominant  
14 region. Contrary to what is speculated by the conventional  
15 wisdom, it is found preferably to have peptides with longer  
16 amino acid chains, ideally longer than 20, synthesized in order  
17 to optimally present these antigenic determinants to HCV  
18 antibodies.

19 Based on the above-mentioned epitope mapping study,  
20 four representative EIAs using peptide IIG alone, or a mixture  
21 of peptides IIF and IID, or a mixture of IIF, IID and V, or a  
22 mixture of IIF and V as the solid phase antigen were  
23 configured for the following efficacy studies as demonstrated  
24 in Examples 2, 8, 9, 10 and 12.

#### 25 EXAMPLE 2

26 Detection of Antibodies to HCV by an  
27 Enzyme-Linked Immunosorbent Assay

28  
29 Wells of 96-well plates were coated at 4°C overnight  
30 (or for 1 hour at 37°C) with either Peptide IIG alone at a

1 coating concentration of 0.5ug per well (designated as IIG EIA)  
2 or with a mixture of two Peptider: IIF and IIID (designated as  
3 IIF/IIID EIA) in a ratio by weight of IIF:IIID=1:1 at 1 ug per  
4 well of the mixture in 100 uL 10mM NaHCO<sub>3</sub> buffer pH 9.5. The  
5 peptide coated wells were then incubated with 250 uL of 3% by  
6 weight of gelatin in PBS at 37°C for 1 hour to block  
7 non-specific protein binding sites, followed by three more  
8 washes with PBS containing 0.05% by volume of TWEEN 20 and  
9 dried.

10 The test specimens were diluted with PBS containing  
11 20% by volume normal goat serum, 1% by weight gelatin and 0.05%  
12 by volume TWEEN 20 at dilutions of 1:20 volume to volume,  
13 respectively. 200 uL of the diluted specimens were added to  
14 each of the wells and allowed to react for 15 minutes at 37°.

15 The wells were then washed six times with 0.05% by  
16 volume TWEEN 20 in PBS in order to remove unbound antibodies.  
17 Horseradish peroxidase conjugated goat anti-human IgG was used  
18 as a second antibody tracer to bind with the HCV  
19 antibody-peptide antigen complex formed in positive wells. 100  
20 uL of peroxidase labeled goat anti-human IgG at a dilution of  
21 1:1800 in 1% by volume normal goat serum, 0.05% by volume TWEEN  
22 20 in PBS was added to each well and incubated at 37°C for  
23 another 15 minutes.

24 The wells were washed six times with 0.05% by volume  
25 TWEEN 20 in PBS to remove unbound antibody and reacted with 100  
26 uL of the substrate mixture containing 0.04% by weight  
27 orthophenylenediamine (OPD) and 0.12% by volume hydrogen  
28 peroxide in sodium citrate buffer, pH 5.0. This substrate  
29 mixture was used to detect the peroxidase label by forming a  
30 colored product. Reactions were stopped by the addition of 100

1     $\mu\text{L}$  of 1.0N  $\text{H}_2\text{SO}_4$  and the absorbance measured using an ELISA  
2    reader at 492nm (i.e.  $A_{492}$ ). Assays were performed in  
3    singlet at one specimen dilution (1:20) with all test  
4    specimens. Each plate run is accompanied by a panel of eight  
5    controls including the specimen diluent blank, negative, weak  
6    HCV reactive and strong HCV reactive controls, all in  
7    duplicate. The strongly reactive control was adjusted by  
8    diluting a HCV positive serum in the specimen dilution buffer  
9    at 1:300, which gave an absorbance value at 492nm of about 1.5  
10    when performed in this standard 45 minute assay procedure. A  
11    cutoff value is calculated based on the following formula:  
12     $\text{Cutoff} = (0.1 \times \text{SRC}) + \text{NRC}$ . Both the raw absorbance  
13    (designated as signal) and the ratio of signal to cutoff are  
14    recorded for all specimens analyzed.

15    The following groups of specimens were analyzed on the  
16    HCV peptide based EIA according to the present invention, with  
17    the plates coated either with 5  $\mu\text{g}/\text{mL}$  of peptide IIG or a  
18    mixture containing 5  $\mu\text{g}/\text{mL}$  IIF and 5  $\mu\text{g}/\text{mL}$  IID:

- 19    (a)    A well-characterized HCV antibody positive control  
20           based on serum dilutions; (on both IIG and IIF/IID  
21           EIAs)  
22    (b)    a panel of serum specimens derived from serial  
23           bleedings of a single individual spanning a period of  
24           sero-conversion to anti-HCV reactivity; (on both IIG  
25           and IIF/IID plates)  
26    (c)    264 normal serum and 264 normal plasma specimens from  
27           commercial sources; (on IIG plates only)  
28    (d)    individuals positive for HBsAg, (n=30); (on both IIG  
29           and IIF/IID plates)  
30    (e)    individuals positive for antibodies to HBe protein,  
         (n=39); (on both IIG and IIF/IID plates)

- 1 (f) individuals with elevated ( $>100$  I.U./L) alanine  
2 aminotransferase (ALT) enzyme activity, (n=174); (on  
3 both IIG and IIF/IIID plates)  
4 (g) individuals positive for antibodies to retroviruses  
5 HIV-1(n=100), HIV-2(n=10), HTLV-1/II(n=14); all  
6 asymptomatic, (total n=124); (on both IIG and IIF/IIID  
7 plates)  
8 (h) individuals with AIDS, ARC(n=200) or ATL (n=170)  
9 disease, (total n=270); (on both IIG and IIF/IIID  
10 plates) and  
11 (i) individuals with autoimmune disease (n=20). (on IIG  
12 plates only)  
13 (j) recombinant SOD/HCV C-100 HCV-EIA repeatedly reactive  
14 specimens obtained from a random donor population,  
15 (n=23). (on both IIG and IIF/IIID plates).  
16

17 Results obtained from groups (a) and (b) are presented  
18 in Figs. 2-1 and 2-2 respectively (data obtained on IIG plates  
19 only), from group (c) in Figs. 3-1 and 3-2; from groups (d) to  
20 (i) in Fig. 4, from group (j) in Table 3 and Figs. 5 and 6.

21 In brief, as shown in Figs. 2-1 and 2-2; a comparison,  
22 by signal to cutoff ratio, between the peptide based HCV-EIA of  
23 the present invention employing peptide IIG and that of  
24 recombinant SOD/HCV C-100 protein based HCV-EIA produced by  
25 Chiron/Ortho. Similar dilution titers and equal ability to  
26 identify date of sero-conversion, the two parameters indicative  
27 of each assay's sensitivity, are obtained for both assays.  
28 However, the assay according to the present invention is more  
29 sensitive and confers a higher signal to cutoff ratio to its  
30 positive specimens.

Table 3

SAMPLE ID No.	HCV S/C	rDNA		ALT (IU/L)	Anti-HBc (S/C)	OTHER POSITIVES	Peptide HCV-EIA S/C
		RPT S/C	RPT S/C				
1	161	5.33	5.56	36/56	2.10		11
2	180	5.76	5.56	78/56	0.07	HBc, ALT	10
3	174	1.98	2.45	20/56	1.97		0.573
4	517	5.79	5.68	34/56	2.04		11
5	581	1.74	2.75	21/56	1.46		0.172
6	475	0.93	1.33	29/56	1.98		0.135
7	720	5.68	5.68	57/56	0.08	HBc, ALT	13
8	773	5.56	5.88	86/56	2.07	HIV, ALT	8.625
9	797	1.79	4.35	74/56	0.38	HBc, ALT	1.802
10	869	5.66	5.59	35/56	2.45		9.755
11	873	5.66	5.59	24/56	2.34		1.189
12	1003	1.63	1.24	31/56	2.02		0.078
13	1073	5.73	5.59	17/56	0.12	HBc	2.594
14	1099	1.72	1.76	10/56	1.84		0.083
15	1118	5.59	5.79	10/56	0.31	HBc	10.5
16	1116	0.93	1.38	18/56	2.15		0.010
17	1501	5.75	5.67	36/56	1.99		5.349
18	1530	1.27	1.48	23/56	2.30		0.943
19	1557	0.91	1.29	20/56	2.20		0.385
20	1652	2.06	2.64	42/56	1.72		0.135
21	1877	5.59	5.63	65/56	2.16	ALT	4.943
22	1940	1.64	1.47	29/56	2.35		0.052
23	2017	5.60	5.84	11/56	0.19	HBc	6.786

Col. 1,2,3.-Ortho's HCV results in s/c.; Col 5=ALT values over cutoff in IU/L;  
Col. 4=Abbott's Anti-HBc results in s/c where results UNDER 1.00 are POSITIVE  
due the competitive binding principle of this assay.

1 As shown in Figs. 3-1 and 3-2, the frequency  
2 distribution of the HCV-EIA signal to cutoff ratios, using  
3 peptide IIG at 5ug/mL as the coating concentration, that was  
4 obtained with 264 normal serum and 264 normal plasma specimens  
5 for commercial sources suggested a repeatably reactive rate of  
6 5.3% and 3.4% respectively. These percentages are relatively  
7 high compared with those reported in field clinical trials  
8 (usually 0.5-1.0%) using the rDNA HCV C-100 based EIA kit  
9 (Chiron/Ortho). However, in the assay according to the present  
10 invention, the mean s/c ratios for the negative (n=250) and  
11 screened out positive (i.e. n=14) serum specimens are 0.034 and  
12 7.202 respectively; for the negative (n=255) and positive (n=9)  
13 normal plasma specimens the mean ratios are 0.084 and 7.089  
14 respectively. Such a sharp contrast between the screened out  
15 positives and all the negatives probably precludes the  
16 likelihood of a high false positive rate. Since these normal  
17 specimens are derived from commercial plasma centers where the  
18 paid donors usually represent a population with higher  
19 incidence of viral markers than the rigorously monitored blood  
20 banks, a higher repeatably reactive rate is also considered  
21 reasonable. Previous clinical studies indicated that between 7  
22 to 10 percent of patients receiving transfusions developed  
23 NANBH, where 90% of these post-transfusion hepatitis cases are  
24 caused by the NANBHV(5). These reports also provide some  
25 support to the interpretation of the data obtained herein that  
26 a high reactivity represents a true positive result.

27  
28 Results obtained from the screening of a total of 677  
29 well-characterized clinical specimens previously categorized  
30 into six groups, from (d) to (i) using a representative lot of  
plates coated with Peptide IIG, were plotted on a histogram as  
shown in Fig. 4.

1           Fifteen out of fifty (i.e. 30%) HBsAg carriers, 3 out  
2 of 39 (i.e. 8%) HBe antibody positive individuals, 43 out of  
3 174 (i.e. 24.7%) individuals with elevated ALT enzyme activity,  
4 8 out of 124 (6.5%) asymptomatic individuals with retroviral  
5 antibodies, 6 out of 270 (i.e. 2.2%) individuals with  
6 retroviral related disease, and 0 out of 20 (i.e. 0%)  
7 individuals with autoimmune disease were found to be repeatably  
8 reactive with the peptide HCV EIA of the present invention  
9 using peptide IIG. All these positive specimens were also  
10 found to be positive when tested on peptides IIF/IIID HCV EIA,  
11 although with much higher s/c ratios.

12           A much higher percentage of positive cases was found  
13 with those who have abnormal liver functions (24.7%) or  
14 previous infection(s) with Hepatitis B (30% and 8%) when  
15 compared to those with other infections or diseases (e.g. 6.5%,  
16 2.2% and 0%).

17       Note: Sera from HBsAg carriers were kindly provided by the  
18 Infectious Diseases Laboratory of the American Red  
19 Cross; sera from HBe antibody positive donors were  
20 obtained from Boston Biomedica Inc.; sera from  
21 individuals with elevated ALT levels (>100 I.U./L)  
22 were obtained from both Boston Biomedica Inc. and NABI  
23 laboratory; sera from asymptomatic individuals with  
24 retroviral antibodies (HIV-1 and HTLV-1) were obtained  
25 from New York Blood Center, and those with HIV-2  
26 antibodies were from Guinea Bissau of West Africa,  
27 kindly provided by Dr. O. Varnier of Italy.; sera from  
28 patients with ATL were kindly provided by the Japanese  
29 Red Cross; sera from patients with AIDS and ARC, were  
30 kindly provided by Dr. D. Knowles at Columbia



1 University College of Physicians and Surgeons, and Dr.  
2 F. Siegal at the Long Island Jewish Hospital; sera  
3 from patients with various complications of autoimmune  
4 diseases were kindly provided by Dr. N. Chiorazzi of  
5 the Cornell University Medical School. All sera have  
6 been characterized by additional licensed serologic  
7 markers before inclusion in the current study.

8 Table 3 illustrates results obtained with the peptide  
9 based HCV EIA described in this invention on a panel of 23  
10 recombinant HCV EIA repeatably reactive specimens obtained from  
11 a random donor population. Data on each specimen's ALT level  
12 and HBe antibody reactivity are provided as supplemental  
13 information for indirect confirmation of NANBH status of the  
14 positive donors. As can be seen from the Table, all eight  
15 specimens with indirect confirmation of their NANBH status  
16 scored positive in the peptide based EIA according to the  
17 present invention (on both IIG and IIF/IIID plates). In  
18 addition, four specimens that scored high on the peptide based  
19 assay also scored as strong positives by the recombinant HCV  
20 EIA, thus further confirming the HCV positivity of these  
21 specimens. Only one specimen scored marginally positive on the  
22 peptide based HCV EIA, which lacks the other markers. However,  
23 this specimen scored positively with the recombinant HCV EIA.  
24 The remaining ten specimens that scored negative by the peptide  
25 based EIA according to the present invention all had a marginal  
26 s/cutoff ratio of between 0.9 to 2.6. Fig. 5 provides a direct  
27 correlation between the peptide based HCV EIA of the present  
28 invention and the recombinant based HCV EIA by their respective  
29 s/cutoff ratios for this panel. Thus, the peptide based HCV  
30 EIA of the present invention can clearly differentiate the

1 repeatedly reactive specimens previously screened out by the  
2 rDNA based HCV EIA into two distinct groups, a positive group  
3 which correlated highly to those with other known NANBH markers  
4 and a negative group which probably represents specimens with  
5 extraneous reactivities unrelated to HCV. In addition to its  
6 use as a screening assay, the peptide based HCV EIA may also  
7 function as a positive confirmatory test for the rDNA based HCV  
8 EIA.  
9

10 Note: This well-characterized serum panel was kindly  
11 provided by Dr. C. Fang of the American Red Cross QC  
12 laboratory.  
13

#### 14 EXAMPLE 1

##### 15 Detection of Antibodies to HCV 16 By an Agglutination Based Assay

17 The presently claimed HCV peptides, synthesized  
18 according to the Merrifield solid phase method, can be  
19 conjugated to bovine serum albumin (BSA) by a simple  
20 crosslinking method in the presence of a low percentage of  
21 glutaraldehyde solution (0.025%), or with other crosslinking  
22 reagents such as m-maleimidobenzoyl-N-hydroxysuccinimide ester  
23 (MBS) according to a previously published procedure  
24 (Biochemistry, 18:690-697, 1979). For example: to 0.32 mL of  
25 a BSA solution (10 mg/mL in 0.01 M phosphate buffer, pH 7.0) at  
26 room temperature is added 0.013 mL of an MBS solution (0.025  
27 mg/mL in dimethylformamide). The amount of MBS added to the  
28 BSA solution can be varied dependent on the optimal molar ratio  
29 of BSA to MBS determined for a specific conjugate studied. The  
30 mixture is stirred at room temperature for 1 hour, after which

1 it is centrifuged to remove any precipitated albumin. The  
2 clarified mixture is then subjected to gel filtration on  
3 Sephadex G-25 and the protein-containing fractions, as detected  
4 by their absorbance at 280 nm, are pooled and stored frozen at  
5 -70°C until needed.

6 The peptides are dissolved in H<sub>2</sub>O at 10 mg/mL. A  
7 predetermined amount of each peptide solution is added dropwise  
8 to the previously activated BSA-MBS solution and stirred at  
9 room temperature for 3 hours. The final peptide-BSA conjugates  
10 are separated from other free peptides by gel filtration or  
11 extensive dialysis. The ratio of peptide to BSA is determined  
12 by SDS-PAGE according to conventional methods.

13 Using the above mentioned peptide-BSA conjugation  
14 process, conjugated peptide 11G-BSA was absorbed to double  
15 aldehyde fixed human O erythrocytes at pH 4.0. The  
16 peptide-conjugate coated erythrocytes were then treated with  
17 NaBH<sub>4</sub> to prevent non-specific protein binding. The  
18 peptide-conjugate coated erythrocytes were then washed with PBS  
19 and incubated with 5% normal human serum-PBS solution. These  
20 processed cells were then used in an agglutination assay for  
21 the detection of HCV antibodies in both serum and plasma  
22 specimens. The specimens were diluted 1:10 in a sample diluent  
23 buffer and an equal volume of the indicator cells (50 ul.) was  
24 mixed with the diluted specimens. The agglutination pattern  
25 was settled within one hour; and the assay results were read by  
26 the naked eye and further quantitated by an optical device  
27 (manufactured by Olympus Corporation) which gave a P/C ratio,  
28 as determined by the absorbance readings of the periphery and  
29 center of the wells. In this experiment, a P/C ratio of 20 was  
30 set as the assay cutoff value, i.e. a positive agglutination  
pattern had a ratio of <20 and a negative pattern, >20.

1 A total of 20 rDNA HCV EIA repeatably reactive  
2 specimens were tested for antibodies to HCV in the  
3 above-described HCV passive hemagglutination assay (PHA)  
4 employing Peptide IIG-BSA conjugate as the solid phase. Figure  
5 6 provides a correlation study between the peptide based HCV  
6 PHA and the recombinant based HCV EIA by their respective P/C  
7 and s/c ratios. All samples with s/c EIA ratios higher than 3  
8 were found to be positive with the HCV PHA test. With the  
9 exception of one, all specimens having borderline s/c ratios  
10 (between 0.9 to 2) scored as negative in this PHA test.  
11

#### 12 EXAMPLE 4

13 Detection of Antibodies to HCV By An  
14 Agglutination Assay Utilizing As the Solid Phase  
15 Immunosorbent Gelatin Particles, Erythrocytes  
16 of Different Animal Species, Or Latex Particles  
17 Coated with a Mixture of HCV Peptides

18 One mL thoroughly washed erythrocytes, gelatin  
19 particles, or polystyrene latex particles are coated with the  
20 HCV peptide mixture, or conjugates thereof at an effective  
21 concentration. The peptide mixture, or conjugates thereof,  
22 coated cells or particles are then incubated with serially  
23 diluted serum samples in the wells of a 96-well U-shaped  
24 microplate or on a slide. After being left at room temperature  
25 for about an hour, or a few minutes in the case of latex  
26 particle based microagglutination, the settled agglutination  
27 pattern on the bottom of each well or on the slide is read; and  
28 the highest dilution showing a positive reaction is recorded.

29 This is a one-step assay which can be used for both  
30 qualitative and quantitative detection of antibodies to HCV in  
specimens including sera or biofluids.

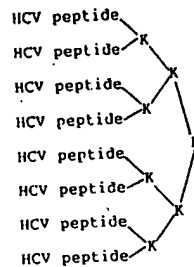
## 1

2

1

1





Octameric HCV peptides of the present invention (Table 1) using the solid phase method of Merrifield are synthesized by an automated peptide synthesizer, either Applied Biosystems (ABI) Model 430A, or Biosearch Model 9500.

Both acid-labile tert-butyloxycarbonyl (t-Boc) and acid-stable groups are used for the protection of N- $\alpha$  amino acid and the functional side chains of the amino acids during the synthesis, respectively. The octameric peptides are synthesized by coupling onto a synthetic octamer resin.

An octamer resin is prepared by coupling di-t-Boc Lys onto 0.14 mmol/g MBHA (4-Methyl benzhydrylamine) resin. (Biosearch 9500 is used for this preparation due to its flexibility in scale). Di-Boc Lys single coupling is followed by two capping reactions (e.g. 0.3M Acetylimidazole in DMF dimethylformamide). The substitution level of synthetic octamer resin is determined by Ninhydrin Test.

Duncan Hartly random bred female guinea pigs (two per immunogen), weighing 400-500 gms, are used as the hosts. For initial immunizations, an aliquot of 100 ug octameric HCV peptide in 0.5 mL PBS is mixed with an equal volume of complete Freund's adjuvant and injected into each animal both

1 subcutaneously and intradermally over multiple sites. After  
2 two to three weeks of rest, an identical dosage of the same  
3 immunogen is given as a boost into each animal except that  
4 incomplete Freund's adjuvant is used. The animals are bled by  
5 heart puncture periodically to monitor each serum's anti-HCV  
6 titers. Subsequent booster shots are given periodically.

#### 8 EXAMPLE 8

9 Relative (%) Immunoreactivity for  
10 Synthetic Peptides By An  
Enzyme-Linked Immunosorbent Assay

11 Wells of 96-well plates were coated at 4°C overnight  
12 (or 1 hour at 37°C), with each of the additional nine peptides,  
13 VA, VB, VC, VD, VE (-V), VIA, VIB, VIC, VID, VIE (see Table 1  
14 for the above mentioned peptides), at 5 ug/mL at 100 uL per  
15 well in 10mM NaHCO<sub>3</sub> buffer, pH 9.5. Each peptide's  
16 immunoreactivity was measured as previously described in  
17 Example 1. Results obtained for the 10 peptides in the V and  
18 VI series are shown in Table 2. According to the EIA  
19 absorbance readings at 492nm (y axis) and the amino acid  
20 sequences for each of the corresponding HCV peptides (x axis),  
21 representative immunoreactivity profiles are plotted for four  
22 of the eight sera on the 10 peptides in the V and VI series,  
23 together with the first twenty peptides in the I, II and III  
24 series, as shown in Figures 1-1 to 1-4. Relative (%)  
25 immunoreactivity index for each of the additional 10 peptides  
26 is likewise calculated using peptide IIID as a reference.  
27 Additional clusters of residues, such as ASRQA and EVIAP, that  
28 are identified with these 10 peptides, were found to contribute  
29 additionally to the overall HCV antibody reactivity.  
30



1 In summary, epitope mapping analysis conducted with a  
2 series of overlapping peptides reveals a varying degree of  
3 immunoreactivities between different HCV antibody positive  
4 samples and these HCV peptides. Based on the above-mentioned  
5 epitope mapping study, a third representative EIA using  
6 peptides IIF, IIID and V as the solid phase antigen was also  
7 configured for testing as shown in Example 9 in comparison to  
8 that using peptide IIF and IIID.  
9

#### 10 EXAMPLE 2

##### 11 Detection of Antibodies to HCV in Serial Samples 12 by Enzyme-Linked Immunosorbent Assays

13 (a) A coded panel consisting of 24 samples derived  
14 from a case of transfusion transmitted NANBH were tested in two  
15 types of EIAs using plates coated with either a mixture of IIF  
16 and IIID at 5.5 ug/mL or a mixture of IIF, IIID and V at 5.5, 5  
17 ug/mL. The panel was provided by Dr. H. Alter of NIH and the  
18 results were decoded by his laboratory.

19 As shown in Figure 7-1, the two anti-HCV profiles, as  
20 tested by two formats, using Peptides IIF/IIID/V coated plate  
21 (Curve A) and Peptides IIF/IIID coated plate (curve B)  
22 respectively, spanning a ten year period revealed an  
23 interesting contrast.

24 According to the record, the seronegative patient  
25 received HCV contaminated blood units on August 20, 1980. As a  
26 result of the transfusion, a trace amount of passive HCV  
27 antibodies was detected in the recipient's serum by format A.  
28 Active development of HCV antibodies by the recipient became  
29 detectable by both formats from November 14th on (about three  
30 months after the initial transfusion). The HCV antibodies,

1 developed as a result of HCV infection through blood  
2 transfusion, persisted throughout the next ten years. Higher  
3 antibody signals were detected by plates coated with an extra  
4 peptide V (curve A) in sera collected four months after the  
5 transfusion. It appears that the epitope presented by peptide  
6 V, representing a neighboring immunodominant region, elicits  
7 abundant HCV antibodies at a slightly later stage than the  
8 epitopes represented by peptides IIF and IIID.  
9

10 (b) Serial samples from one representative case of a  
11 hemodialysis patient with NANBH were provided by Dr. Cladd  
12 Stevens of New York Blood Center, N.Y., N.Y., and tested on  
13 plates coated with a mixture of three peptides, IIF/IIID/V.  
14 The sample histories are shown in Figure 7-2. The results show  
15 that the peptide based EIA detects samples about two months  
16 after the onset of the acute phase of the disease as evidenced  
17 by the ALT elevation.

18 (c) Serial samples from a representative chimpanzee  
19 were tested with a peptide based HCV EIA using a mixture of  
20 IIF/IIID/V peptides. This chimpanzee was inoculated on day 0  
21 with a well-characterized strain of NANBHIV. Following the  
22 acute phase of infection as evidenced by the rise of the ALT  
23 levels, antibodies to HCV were detected about 60 days after  
24 inoculation [Figure 7-3].

#### 25 EXAMPLE 10

#### 26 Screening of Low Risk Random Blood Donors 27 With the Peptide Based HCV EIA

28 2035 donor specimens obtained in a blood bank setting  
29 were tested by EIA coated with a mixture of Peptides IIF, IIID  
30 and V at 5 ug/mL each following the procedure described in

1 Example 2. The results are shown in Figures 8-1 and 8-2. The  
2 frequency distribution of the peptide based HCV-EIA signal to  
3 cutoff ratios, suggested an initial reactive rate of 1.18% and  
4 a repeatably reactive rate of 1.08 respectively. 88% of the  
5 initial reactive specimens are repeatably reactive indicating a  
6 high reproducibility of the assay. The repeatably reactive  
7 rate of the peptide based HCV EIA obtained with the low risk  
8 random blood donor specimens, all volunteers, is lower than  
9 that obtained from the commercial paid donor population (See  
10 Example 2).  
11

12 EXAMPLE 11

13 Synthetic Peptide Based HCV Neutralization EIA  
14 As a Confirmatory Test

15 Wells of 96-well plates were coated at 4°C overnight  
16 (or for 1 hour at 37°C) with a mixture of two peptides IIF and  
17 IIID at 5 ug/mL each in 100 uL 10mM NaHCO<sub>3</sub> buffer pH 9.5.  
18 Repeatably reactive specimens previously screened out by the  
19 direct HCV EIA were incubated with either a control specimen  
20 diluent buffer (i.e., PBS containing 20% by volume normal goat  
21 serum, 1% by weight gelatin and 0.05% by volume Tween 20) at a  
22 dilution of 1:20 volume to volume, or with the same specimen  
23 diluent buffer containing varying amounts of a HCV peptide  
24 analogue IV (see Table 2 for its amino acid sequence) and  
25 allowed to react for an hour at 37°.

26 200 uL of the peptide IV neutralized specimens were  
27 then added to each of the wells and allowed to react for 15  
28 minutes at 37°, followed by the EIA procedure as described in  
29 Example 2. Four representative reactive samples including two  
30 weakly reactives and two strongly reactives were tested. One

1 of the strong reactives was further diluted at 1:10 in the  
2 specimen diluent prior to neutralization testing. As shown in  
3 Figure 9 and Table 4, a dose dependent inhibition (or  
4 neutralization) of HCV EIA was observed with peptide IV. When  
5 compared with the controls, a significant inhibition was  
6 obtained with all four specimens even at a concentration of 50  
7 ug/ml peptide IV.

#### 8 EXAMPLE 12

##### 9 Detection of Antibodies to HCV in 10 Hemodialysis patients by EIA

11  
12 A coded panel consisting of 74 samples from a group of  
13 hemodialysis patients was tested in two types of EIAs using  
14 plates coated with a mixture of HCV peptides III and V at 10,5  
15 ug/ml, or a recombinant HCV protein based EIA. The panel was  
16 provided by investigators at the Japanese National Institute of  
17 Health and the results were decoded and compared to the  
18 recombinant HCV protein based EIA by the sera provider.

19 As shown in Figure 10, an x-y plot of the A492 nm  
20 readings for the peptide based HCV EIA and the recombinant HCV  
21 protein based HCV EIA revealed a high correlation between these  
22 two assays. (A cutoff value of 0.2 and 0.4 was obtained based  
23 on the corresponding assay design.) These 74 specimens  
24 obtained from the hemodialysis patients who are highly  
25 susceptible to HCV infection were grouped into four categories  
26 based on their respective reactivities with these two types of  
27 EIAs. The upper right block indicates samples that are scored  
28 positive by both assays, and the lower left block indicates  
29 samples that are scored negative by both assays. None of the  
30 74 high risk samples were found positive by the recombinant

1 based EIA and negative by the peptide based EIA as shown in the  
2 upper left block; whereas five of these 74 high risk samples  
3 reacted positive by the peptide based EIA and negative by the  
4 recombinant based EIA as shown in the lower right block  
5 indicating that the peptide based HCV EIA is more sensitive  
6 when tested with specimens derived from patients at high risks  
7 for HCV infection.

#### 8 EXAMPLE 11

9  
10 Detection Of Anti-HCV Activity In Rare  
11 Specimens With An Elevated ALT Level

12 These results are representative of the acute phase of  
13 HCV infection by synthetic peptides of Peptide VII series,  
14 covering a region near the C-terminus of the HCV protein C-100,  
15 and Peptide IIH from the immunodominant region.

16 Wells or 96-well plates were coated at 37°C for 1 hour  
17 with each of the six peptides VIIA, VIIB, VIIC, VIID, IIG and  
18 IIH, at 5 ug/mL at 100 µL per well in 10mM NaHCO<sub>3</sub> buffer, pH  
19 9.5. Each peptide's immunoreactivity with the respective  
20 specimen was measured as previously described in Example 1. As  
21 shown in in Table 5, weak immunoreactivity was obtained with  
22 specimen 3 for peptides VIIC and VIID, but not VIIA and VIIB.  
23 Moderate immunoreactivity was obtained with specimen HAN-2-2  
24 for peptide IIH, but not IIG. Both specimens were found to  
25 have high ALT level and are representative of specimens from  
26 patients with acute phase of HCV infection.

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TABLE 3  
Peptide Based HCV Neutralization EIA  
As A Confirmatory Test

Specimens		Peptide UV Concentration						Control	
		400 ug/mL		200 ug/mL		100 ug/mL		50 ug/mL	
ID	Dilution	MA	NI	MA	NI	MA	NI	MA	NI
A	1:1	590	72.0	1129	46.5	1066	49.5	1161	35.4
	1:10	244	86.1	325	81.5	409	76.7	510	71.0
B	1:1	161	92.0	209	89.6	321	84.1	521	74.1
	1:1	117	83.0	162	84.7	355	85.4	353	85.6
C	1:1	27	89.8	23	91.3	38	85.7	34	87.2
	1:1								

INHIBITION = MA/control - MA(ug/mL)  
MA (control)

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Table 5

		A492nm
		Specimen No.
Code	Amino Acid Sequence	NAB-2-2 13
11H	SG, KPAT, PDREV, LYREF, DEMEE, CSOHL, PYIEQ, GQHLA, EGFKO, KALGL 1.232(+)	
11G	11, PDREV, LYREF, DEMEE, CSOHL, PYIEQ, GQHLA, EGFKO, KALGL 0.013(-)	
VITA	AVQMM, NRLIA, FASRG, NHVSP	0.109
VIID	RHV, GPCEG, AVQMM, NRLIA, FASRG, NHVSP	0.224
VIIIC	V, VCAAT, LRRHV, GPCEG, AVQMM, NRLIA, FASRG, NHVSP	0.674
VIID	PHA, LVVGV, VCAAT, LRRHV, GPCEG, AVQMM, NRLIA, FASRG, NHVSP	0.658

It is to be understood that the above examples are illustrative of the present invention and are not meant to limit the scope thereof.

1 WE CLAIM:

2

3

4 1. A peptide composition comprising a peptide with  
5 an amino acid sequence selected from the group consisting of:

6 (i) EE, SCQHL, PYIEQ, GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAP-X (I)

7 (ii) II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X (II)

8 (iii) SG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X (III)

9 (iv) CV, VIVGR, VVLSG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYI-X (III)

10 (v) SG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQF-X (IV)

11 (vi) KQ, KALGL, LQTAS, RQAEV, IAPAV, QTNWQ, KLETf, WAKHM, WNF-X (V)

12 (vii) EQ, GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAPAV, QTNWQ, KLET-X (VI)

13 and

14 (viii) PGALV, VGVVC, AAILR, RHVGP, GEGAV, QMMNR, LIAFA, SRGNH, VSP-X (VII)

15 Wherein X is -OH or -NH<sub>2</sub>; and

16 (ix) analogues, segments, mixtures, combinations, conjugates and polymers thereof.

17

18 2. A peptide composition according to Claim 1  
19 comprising a combination of Peptides I, II, III and V and  
20 having the amino acid sequence:

21 CV, VIVGR, VVLSG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYIEQ,

22 GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAPAV, QTNWQ, KLETf, WAKHM,

23 WNF-X

24 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

25

26 3. A peptide composition according to Claim 1

27 comprising a segment of Peptide II and having an amino acid  
28 sequence selected from the group consisting of:

29

30



1 (i) CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
 2 (ii) DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
 3 (iii) LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
 4 (iv) PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
 5 KALGL-X;  
 6 (v) II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
 7 KALGL-X;  
 8 (vi) SG, KPAII, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
 9 KALGL-X;  
 10 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.  
 11 4. A peptide composition according to Claim 3 and  
 12 having an amino acid sequence as follows:  
 13 SG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
 14 KALGL-X  
 15 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
 16 5. A peptide composition according to Claim 1  
 17 comprising a segment of Peptide III and having an amino acid  
 18 sequence selected from the group consisting of:  
 19 (i) II, PDREV, LYREF, DEMEE, CSQHL, PYI-X;  
 20 (ii) SG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYI-X;  
 21 (iii) GR, VVLSG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYI-X;  
 22 (iv) CV, VIVGR, VVLSG, KPAII, PDREV, LYREF, DEMEE, CSQHL,  
 23 PYI-X  
 24 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.  
 25 6. A peptide composition according to Claim 5 and  
 26 having an amino acid sequence as follows:  
 27 SG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYI-X  
 28 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
 29  
 30

1 7. A method of detecting, in body fluids, antibodies  
2 to hepatitis C virus (HCV) or diagnosis of HCV infection or  
3 NANBH comprising the steps:

- 4 (i) Preparing a peptide composition according to  
5 Claim 1;  
6 (ii) Using an effective amount of the peptide  
7 composition as an antigen to form a complex  
8 with antibodies to HCV or NANBH;  
9 (iii) Detecting the presence of the complex of  
10 peptide with antibodies to HCV or NANBH by an  
11 enzyme linked immunosorbent assay, an  
12 immunoradiometric assay or an agglutination  
13 assay or other immunoassays.  
14

15 8. A method according to Claim 7 where in the  
16 peptide composition is coated on a solid substrate.  
17

18 9. A method according to Claim 8 wherein the step of  
19 detecting the presence of the complex of peptide with  
20 antibodies to HCV or NANBH is by means of an enzyme linked  
21 immunosorbent assay.  
22

23 10. A method according to Claim 7 wherein the method  
24 of detecting the presence of the complex of peptide with  
25 antibodies to HCV or NANBH is by using an immunoradiometric-  
26 assay.  
27

28 11. A method according to Claim 7 wherein the method  
29 of detecting the presence of the complex of peptide with  
30 antibodies to HCV or NANBH is by an agglutination assay.

1 12. A method according to Claim 7 wherein the peptide  
2 composition is a combination of peptides I, II, III and V and  
3 having the amino acid sequence:

4 CV,VIVGR,VVLGG,KPAII,PDREV,LYREF,DEMEE,CSQHL, PYIEQ,  
5 GMMLA, EQFKQ, KALGL, LQTAS, RQAEV, IAPAV, QTNWQ, KLETF, WAKHM,  
6 WNF-X  
7 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

8 13. A method according to claim 7 wherein the peptide  
9 composition comprises a segment of Peptide II and has an amino  
10 acid sequence selected from the group consisting of:

- 11 (i) CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
12 (ii) DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
13 (iii) LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
14 (iv) PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
15 KALGL-X;  
16 (v) II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
17 KALGL-X  
18 (vi) SG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA,  
19 EQFKQ, KALGL-X  
20 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

21 14. A method according to claim 7, wherein the  
22 peptide composition comprises a peptide having an amino acid  
23 sequence:

24 PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X  
25 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

26 15. A method according to claim 7 wherein the peptide  
27 composition is a segment of peptide III and having an amino  
28 acid sequence selected from the group consisting of:

- 29 (i) II, PDREV, LYREF, DEMEE, CSQHL, PYI-X;  
30 (ii) SG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYI-X;

1 (iii) GR,VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQKL,PLYI-X;  
2 (iv) CV,VIVGR,VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQKL,  
3 PLYI-X;  
4 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

5 16. A method according to claim 7 wherein the peptide  
6 composition comprises a peptide having an amino acid sequence:  
7 SG,KPAII,PDREV,LYREF,DEMEE,CSQKL,CSQKL,PLYI-X  
8 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

9  
10 17. Antibodies to HCV or NANBHV produced by using as  
11 an immunogen a peptide composition comprising a peptide with an  
12 amino acid sequence selected from the group consisting of:

- 13  
14 (i) EE,SCQHL,PLYEQ,GMMLA,EQFKQ,KALGL,LQTAS,RQAEV,IAP-X (I)  
15 (ii) II,PDREV,LYREF,DEMEE,CSQHL,PLYEQ,GMMLA,EQFKQ,KALGL-X (II)  
16 (iii) SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PLYEQ,GMMLA,EQFKQ,  
17 KALGL-X (III)  
18 (iv) CV,VIVGR,VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PLYI-X (IV)  
19 (v) SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PLYEQ,GMMLA,EQF-X (V)  
20 (vi) KQ,KALCL,LQTAS,RQAEV,IAPAV,QTNWQ,KLETF,WAKHM,WNF-X (VI)  
21 (vii) EQ,GMMLA,EQFKQ,KALGL,LQTAS,RQAEV,IAPAV,QTNWQ,KLET-X (VII)  
22 and  
23 (viii) PGALV,VGVVC,AAILR,RHVGF,SEGAV,QNMHR,LIAFA,SCQHL,  
24 VSP-X (VIII)

25 wherein X is -OH or -NH<sub>2</sub>; and

26 (ix) analogues, segments, mixtures, combinations,  
27 conjugates and polymers thereof.

28 18. Antibodies to HCV or NANBHV according to claim 17  
29 wherein the immunogen comprises a combination of Peptides I,  
30 II, III and V and having the amino acid sequence:  
CV,VIVGR,VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PLYEQ,  
GMMLA,EQFKQ,KALGL,LQTAS,RQAEV,IAPAV,QTNWQ,KLETF,WAKHM,  
WNF-X

1 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

2

3 19. Antibodies to HCV or HANRHV according to claim 17  
4 wherein the immunogen comprises a segment of Peptide II and  
5 having an amino acid sequence selected from the group  
6 consisting of:

- 7 (i) CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
8 (ii) DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
9 (iii) LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
10 (iv) PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
11 KALGL-X;  
12 (v) II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
13 KALGL-X;  
14 (vi) SG, KPAII, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
15 KALGL-X;

16 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

17 20. Antibodies to HCV or NANBHV according to claim 19  
18 wherein the immunogen comprises a peptide composition having an  
19 amino acid sequence as follows:

20 PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X

21 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

22 21. Antibodies to HCV or NANBHV according to claim 17  
23 wherein the immunogen comprises a segment of Peptide III and  
24 having an amino acid sequence selected from the group  
25 consisting of:

- 26 (i) II, PDREV, LYREF, DEMEE, CSQKL, PYI-X;  
27 (ii) SG, KPAII, PDREV, LYREF, DEMEE, CSQKL, PYI-X;  
28 (iii) GR, VVLGS, KPAII, PDREV, LYREF, DEMEE, CSQKL, PYI-X;  
29 (iv) CV, VIVGR, VVLGS, KPAII, PDREV, LYREF, DEMEE, CSQHL,  
30 PYI-X

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

1 22. Antibodies to HCV or NANBHV according to claim 21  
2 wherein the immunogen comprises a peptide composition having an  
3 amino acid sequence as follows:  
4 SG,KPAII,PDREV,LYREF,DEMEE,CSQKL,PYI-X  
5 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
6

7 23. A vaccine composition to prevent HCV infection or  
8 NANBH by using, as an immunogen, a peptide composition  
9 comprising a peptide with an amino acid sequence selected from  
10 the group consisting of:  
11

12 (i) EE,SCQHL,PYIEQ,GMMLA,EQFKQ,KALGL,LQTAS,RQAEV,IAP-X (I)  
13 (ii) II,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL-X (II)  
14 (iii) SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,  
15 KALGL-X (III)  
16 (iv) CV,VIVGR,VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYI-X (III)  
17 (v) SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQF-X (IV)  
18 (vi) KQ,KALGL,LQTAS,RQAEV,IAPAV,QTNWQ,KLETf,WAKHM,WNF-X (V)  
19 (vii) EQ,GMMLA,EQFKQ,KALGL,LQTAS,RQAEV,IAPAV,QTNWQ,KLET-X (VI)  
20 and  
21 (viii) PGALV,VGVVC,AAILR,RHVGP,GEGAV,QWMNR,LIAFA,SRGNH,  
22 VSP-X (VII)  
23 wherein X is -OH or -NH<sub>2</sub>; and  
24 (ix) analogues, segments, mixtures, combinations,  
25 conjugates and polymers thereof.  
26

27 24. A vaccine composition according to claim 23,  
28 wherein the immunogen comprises a combination of Peptides I,  
29 II, III and V and having the amino acid sequence:  
30 CV,VIVGR,VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,  
GMMLA,EQFKQ,KALGL,LQTAS,RQAEV,IAPAV,QTNWQ,KLETf,WAKHM,  
WNF-X  
wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

1 25. A vaccine composition according to claim 23.  
2 wherein the immunogen comprises a segment of Peptide II and  
3 having an amino acid sequence selected from the group  
4 consisting of:

- 5 (i) CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
6 (ii) DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
7 (iii) LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
8 (iv) PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
9 KALGL-X;  
10 (v) II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
11 KALGL-X;  
12 (vi) SG, KPAIL, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
13 KALGL-X;

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

14 26. A vaccine composition according to claim 25,  
15 wherein the immunogen comprises having an amino acid sequence  
16 as follows:

17 PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X

18 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
19

20 27. A vaccine composition according to claim 23,  
21 wherein the immunogen comprises a segment of Peptide III and  
22 having an amino acid sequence selected from the group  
23 consisting of:

- 24 (i) II, PDREV, LYREF, DEMEE, CSQHL, PYI-X;  
25 (ii) SG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYI-X;  
26 (iii) GR, VVLGS, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYI-X;  
27 (iv) CV, VIVGR, VVLGS, KPAIL, PDREV, LYREF, DEMEE, CSQHL,  
28 PYI-X

29 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.  
30

1 28. A vaccine composition according to claim 27,  
2 wherein the immunogen comprises an amino acid sequence as  
3 follows:

4 SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PLY-X

5 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
6

7 29. An enzyme linked immunosorbent assay (ELISA) test  
8 kit for the detection of antibodies to HCV or NANBHV or the  
9 diagnosis of HCV or NANBHV infection comprising:

- 10 (i) compartmented enclosure containing multiple  
11 wells coated with a peptide composition  
according to claim 1;  
12 (ii) a negative control sample;  
13 (iii) an inactivated HCV positive control sample;  
14 (iv) specimen diluent comprising PBS buffer  
15 containing 20% by volume normal goat serum; 1%  
by weight gelatin and 0.05% by weight TWEEN 20;  
16 (v) peroxidase labelled antibodies to human IgG; and  
17 (vi) a color change indicator.  
18

19 30. An ELISA test kit according to claim 29 wherein  
20 the multiple wells are coated with a peptide composition which  
21 is a combination of Peptide I, II, III and V with an amino acid  
22 sequence:

23 CV,VIVGR,VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PLYEQ,  
24 GMMLA,EQEKQ,KALGL,LOTAS,ROAEV,IAPAV,QTNWQ,KLETQ,WAKHM,  
25 WNF-X

26 wherein X is -OH or -NH<sub>2</sub> or analogues thereof.  
27

28 31. An ELISA test kit according to claim 29 wherein  
29 the multiple wells are coated with a peptide composition  
30 comprising a segment of Peptide II and having an amino acid  
sequence selected from the group consisting of:



- 1 (i) CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
2 (ii) DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
3 (iii) LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X;  
4 (iv) PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
5 KALGL-X;  
6 (v) II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
7 KALGL-X;  
8 (vi) SG, KPAII, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ,  
9 KALGL-X;  
10 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

11 32. An ELISA test kit according to claim 29 wherein  
12 the multiple wells are coated with a peptide having an amino  
13 acid sequence:

14 PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL-X  
15 wherein X is OH or -NH<sub>2</sub> or an analogue thereof.

16 33. An ELISA test kit according to claim 29 wherein  
17 the multiple wells are coated with a peptide composition  
18 comprising a segment of Peptide III having an amino acid  
19 sequence selected from the group consisting of:

- 20 (i) II, PDREV, LYREF, DEMEE, CSQHL, PYI-X  
21 (ii) SG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYI-X  
22 (iii) GR, VVLGG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYI-X  
23 (iv) CV, VIVGR, VVLGG, KPAII, PDREV, LYREF, DEMEE, CSQHL,  
24 PYI-X  
25 wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

26 34. An ELISA test kit according to claim 33 wherein  
27 the Peptide is:

28 SG, KPAII, PDREV, LYREF, DEMEE, CSQHL, PYI-X  
29 wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.  
30

1 ABSTRACT

2  
3 The present invention relates to a method for the  
4 detection in body fluids of antibodies to hepatitis C virus  
5 (HCV), also known as a non-A non-B hepatitis (NANBH) virus and  
6 to the diagnosis of NANBH by the use of a composition of  
7 synthetic peptides. Each of these peptides has an amino acid  
8 sequence corresponding to an immunodominant region of a fusion  
9 protein and a polypeptide of HCV, SOD/HCV C100. More  
10 specifically, the present invention is directed to the use of a  
11 group of synthetic peptides in a prescribed sequence or their  
12 analogues for the detection of antibodies to HCV in body  
13 fluids. The detection method includes an enzyme-linked  
14 immunosorbent assay (ELISA), and other forms of immunoassay  
15 procedures.

16 The present invention also relates to a method for  
17 generating high titer antibodies to HCV in healthy mammals,  
18 including humans, by the use of compositions containing these  
19 synthetic peptides, analogues or mixtures thereof, in a free,  
20 conjugated or polymeric form as key components in synthetic  
21 vaccines for the prevention of non-A non-B hepatitis (NANBH).  
22  
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PATENT

Doclet No. 1151-4035

COMBINED DECLARATION AND POWER OF ATTORNEY FOR  
ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL,  
DISCONTINUATION, ABANDONMENT OR OTHER NATIONAL/REG. APPLICATIONS

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below)  
or an original, first and joint inventor (if plural names are listed below) of the  
subject matter which is claimed and for which a patent is sought on the invention  
entitled:

**SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV,  
DIAGNOSIS OF HCV INFECTION, AND PREVENTION THEREOF AS VACCINES**  
the specification of which

a. ☒ is attached hereto

b. ☐ was filed on \_\_\_\_\_ as application Serial No. \_\_\_\_\_  
and was amended on \_\_\_\_\_ (if applicable).

PCT FILED APPLICATION ENTERING NATIONAL STAGE

c. ☐ was described and claimed in International Application  
No. \_\_\_\_\_ filed on \_\_\_\_\_ and as amended  
on \_\_\_\_\_ (if any).

I hereby state that I have reviewed and understand the contents of the above-identified  
specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of  
this application in accordance with Title 37, Code of Federal Regulations, § 1.55(a).

☐ I hereby claim foreign priority benefits under Title 35, United States Code § 119  
of any foreign application(s) for patent or inventor's certificate listed below and have  
also identified below any foreign application for patent or inventor's certificate  
having a filing date before that of the application on which priority is claimed:

☐ The attached 35 U.S.C. § 119 claim for priority for the U.S. application(s) listed  
below forms a part of this declaration.

Application Country Number	Date of filing (day, month, yr)	Date of issue (day, month, yr)	Priority Claimed
			<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO
			<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO
			<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO

PAYMENT

Doctet No. 1151-4035

ADDITIONAL STATEMENTS FOR  
DIVISIONAL CONTINUATION OR CONTINUATION-IN-PART

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) listed below.

07/481,348	February 16, 1990	Pending
Application Serial No.	Filing Date	Status (patented, pending, abandoned)
Application Serial No.	Filing Date	Status (patented, pending, abandoned)

(X) In this continuation-in-part application, insofar as the subject matter of any of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.54(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorneys and/or agents with full power of substitution and revocation, to prosecute this application, to receive the patent, and to transact all business in the Patent and Trademark Office connected therewith: Jerome G. Lee (Reg. No. 16,967), John D. Foley (Reg. No. 16,836), John A. Diaz (Reg. No. 19,550), Thomas P. Dowling (Reg. No. 19,221), John C. Vassil (Reg. No. 19,098), Warren H. Robert (Reg. No. 19,659), Alfred P. Ewert (Reg. No. 19,887), David H. Pfeffer, P.C. (Reg. No. 10,825), Harry C. Marcus (Reg. No. 22,390), Robert E. Paulson (Reg. No. 21,046), Stephen R. Smith (Reg. No. 22,615), Kurt E. Richter (Reg. No. 24,052), J. Robert Dailey (Reg. No. 27,434), Eugene Moroz (Reg. No. 25,237), John F. Sweeney (Reg. No. 27,471), Arnold I. Rody (Reg. No. 26,601), Christopher A. Hughes (Reg. No. 26,914), William S. Feiler (Reg. No. 26,728), Joseph A. Calvaruso (Reg. No. 28,287), James W. Gould (Reg. No. 28,859), Richard C. Komon (Reg. No. 27,913), Israel Blum (Reg. No. 26,710), Bartholomew Verdigrane (Reg. No. 28,483), Maria C. H. Lin (Reg. No. 29,323), and Joseph A. DeGirolamo, (Reg. No. 28,595) of Morgan & Finnegan whose address is: 345 Park Avenue, New York, New York 10154.

[ ] I hereby authorize the U.S. attorneys and/or agents named hereinabove to accept and follow instructions from \_\_\_\_\_

as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and/or agents and me. In the event of a change in the person(s) from whom instructions may be taken I will so notify the U.S. attorneys and/or agents named hereinabove.

82-701

Docket No. 1151-4035

I hereby specify the following as the correspondence address to which all communications about this application are to be directed:

Maria C.H. Lin

SEND CORRESPONDENCE TO:

MORGAN & KENNEDY, 305 Park Avenue, New York, New York 10158

DIRECT TELEPHONE CALLS TO: (212) 415-8520  
(AREA) 755-4500

Full name of sole or first inventor Chang Yi Wang

Inventor's signature: Chang Yi Wang <sup>date</sup> 4/16/90

Residence 159 Hillpark Avenue, Great Neck, New York 11021

Citizenship U.S.A.

Post Office Address: United Biomedical Inc., 2 Nevada Drive, Lake Success  
New York 11042

Full name of second joint inventor, if any \_\_\_\_\_

Inventor's signature \_\_\_\_\_

date \_\_\_\_\_

Residence \_\_\_\_\_

Citizenship \_\_\_\_\_

Post Office Address \_\_\_\_\_

[ ] ATTACHED IS ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY THIRD AND SUBSEQUENT INVENTORS FORM.

\* Before signing this declaration, each person signing must:

1. Review the declaration and verify the correctness of all information therein; and
2. Review the specification and the claims, including any amendments made to the claims.

After the declaration is signed, the specification and claims are not to be altered.

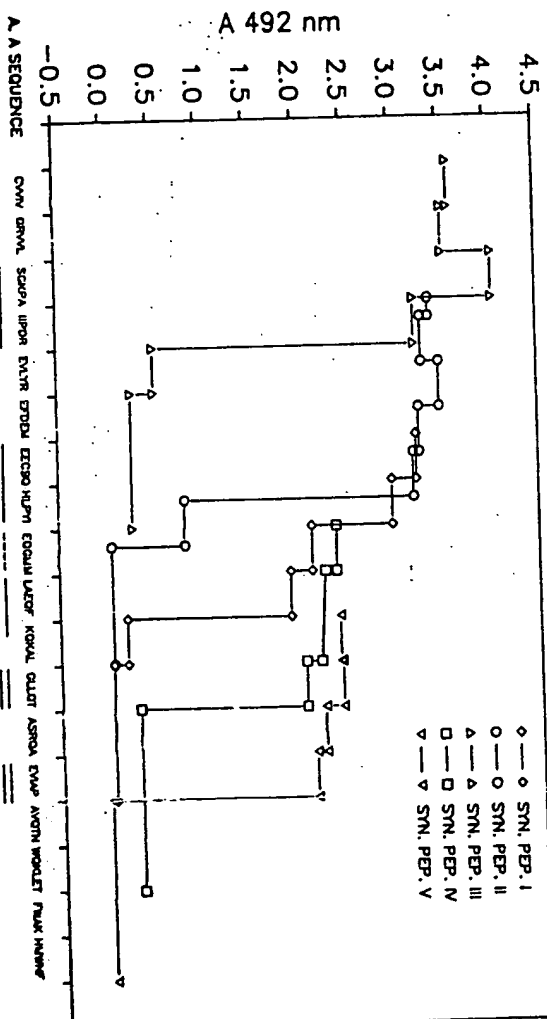
To the inventor(s):

The following are cited in or pertinent to the declaration attached to the accompanying application:

07/510153

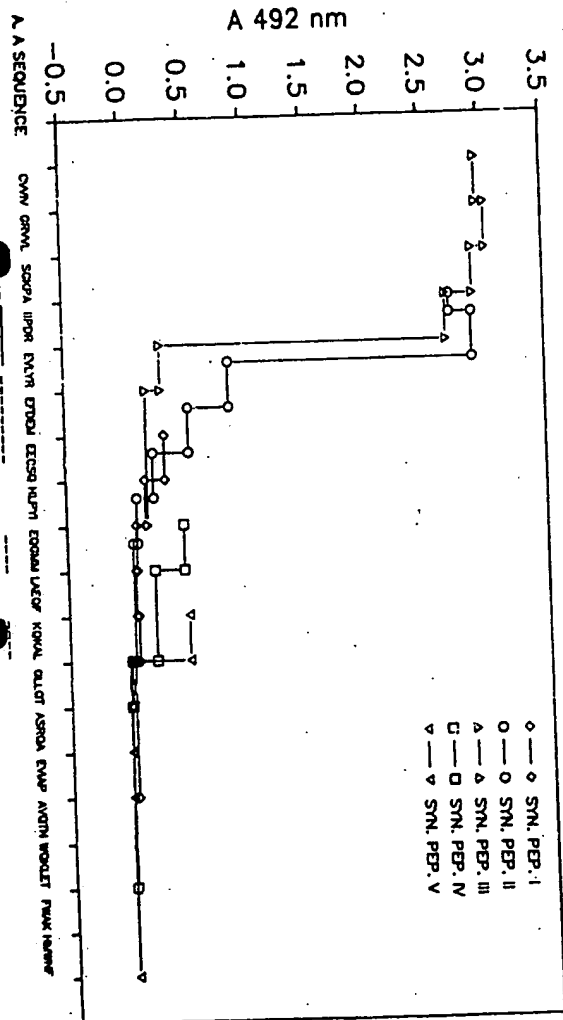
Fig 1-1

EPITOPE MAPPING OF AN IMMUNODOMINANT REGION OF  
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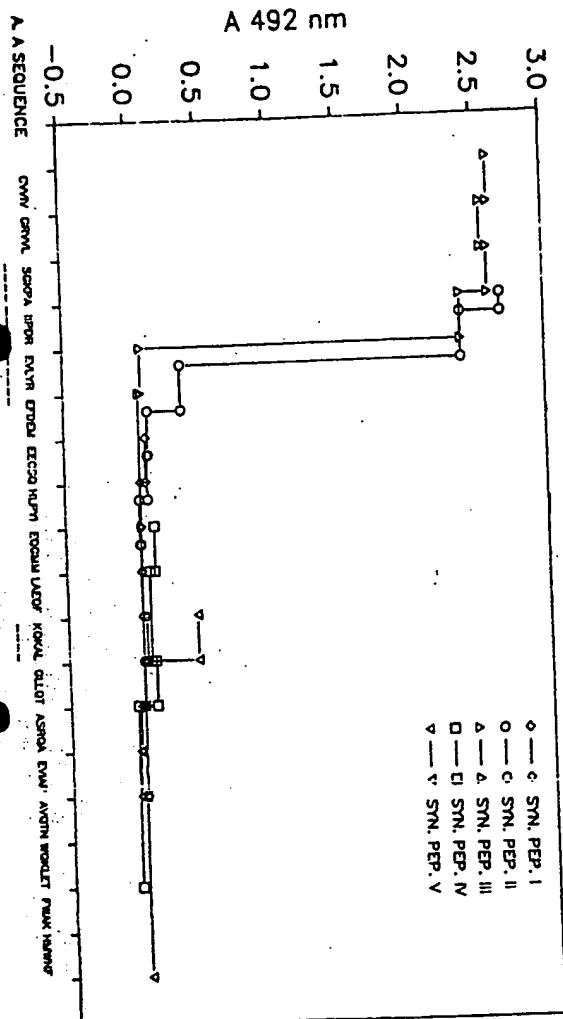
07/510153

7/29/1-2  
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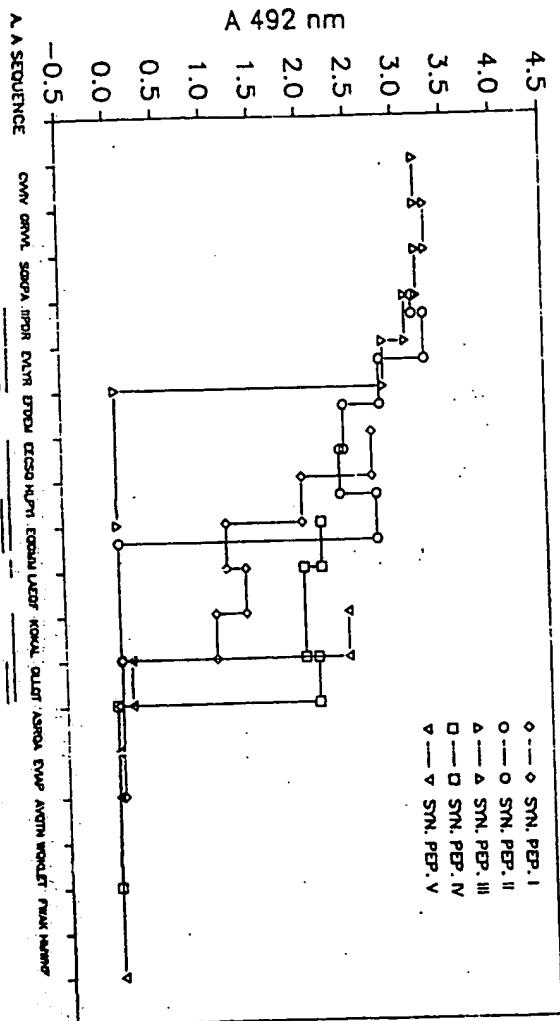


7/510153

*Fig 1-3*  
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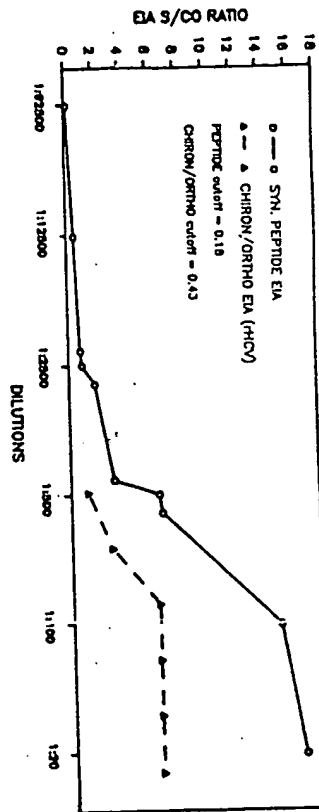




07/510153

Fig 2-1

# COMPARISON of SYN. PEPTIDE VS rDNA PRODUCED HCV EIA



## HCV SEROCONVERSION PANEL

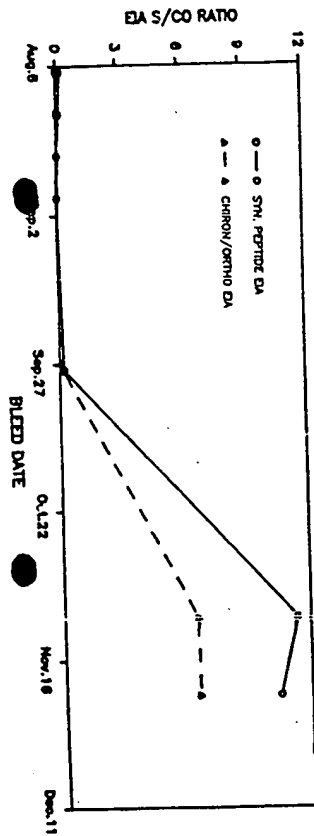


Fig 2-2

7/510153

Fig 3-1

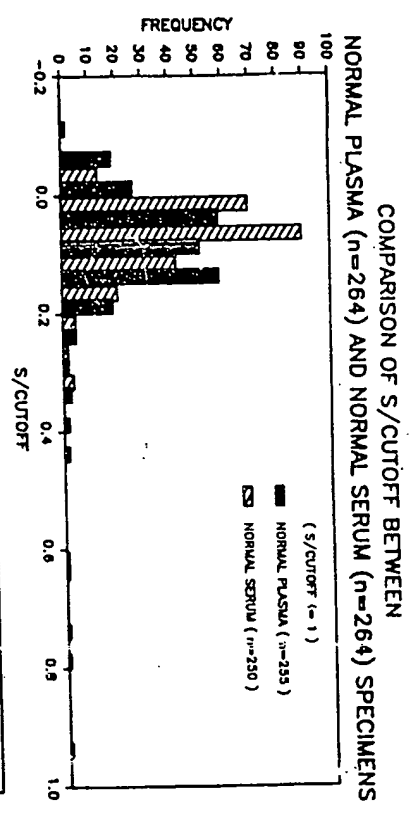
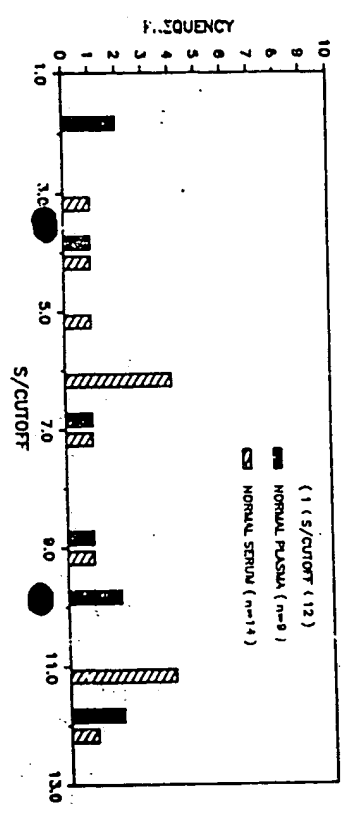


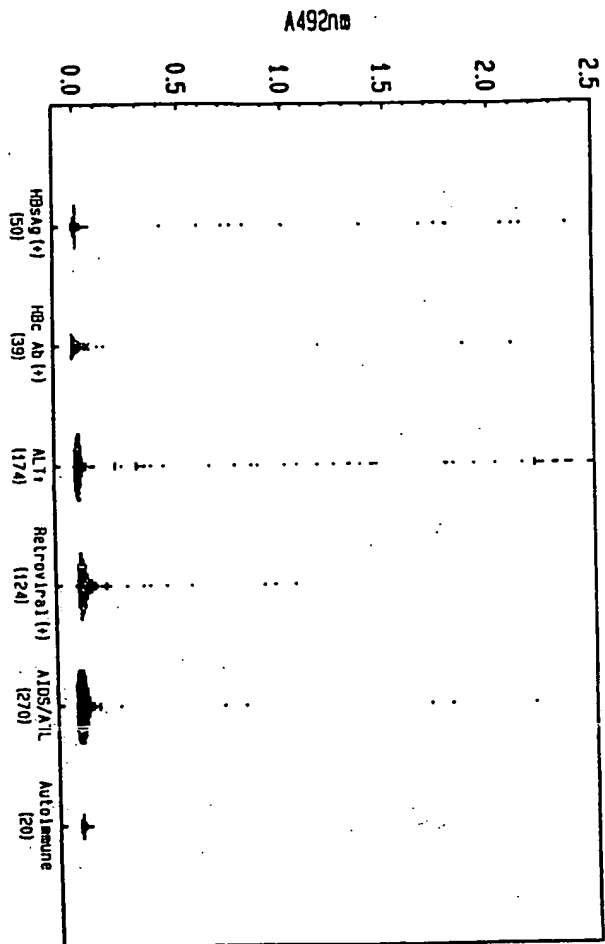
Fig 3-2



07/510153

Fig 4

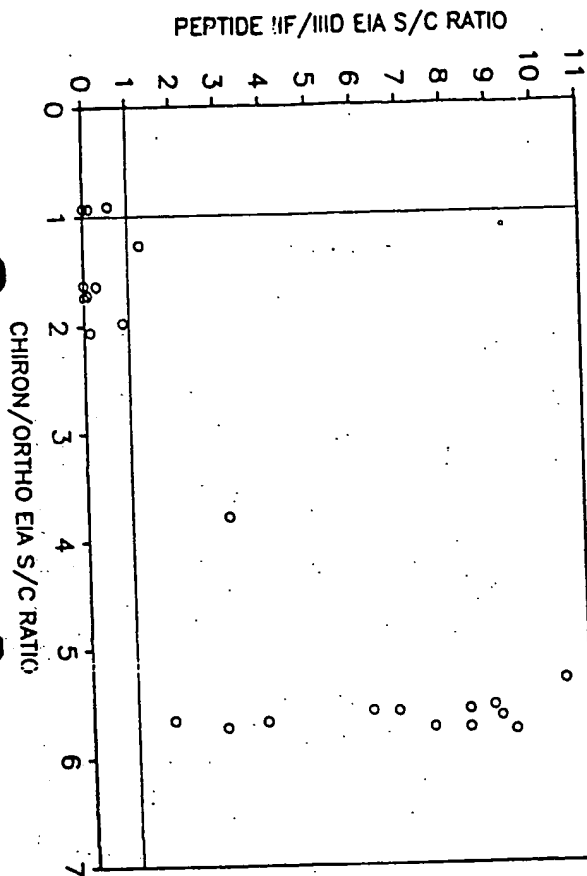
ANTIBODY RESPONSES TO SYNTHETIC HCV PEPTIDE  
IN VARIOUS CLINICAL POPULATIONS



07/510153

Fig 5

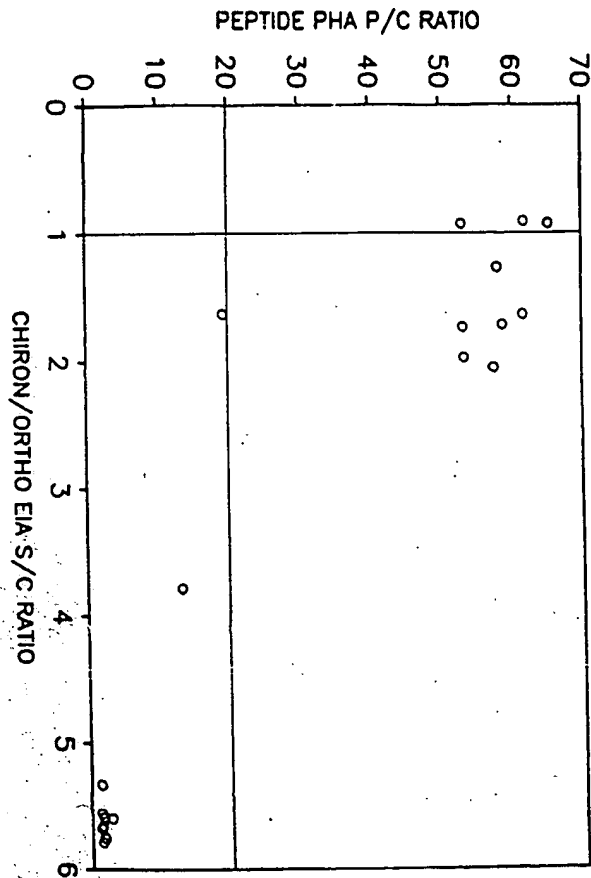
COMPARISON of PEPTIDE IIF/IIID and CHIRON/ORTHO HCV EIAs



01/010153

1.5/5/96

COMPARISON of PEPTIDE HCV PHA and CHIRON/ORTHO HCV EIA

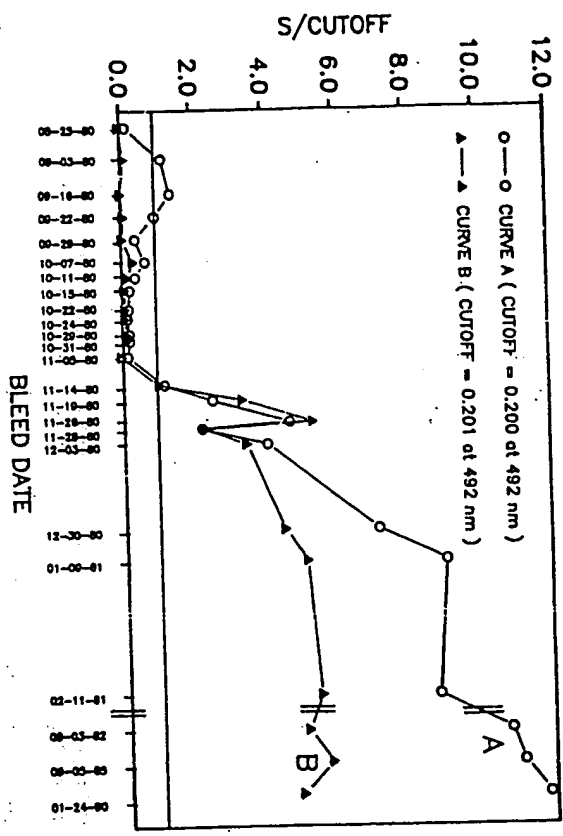


01/510153

1/1/87

# SEROCONVERSION PANEL I

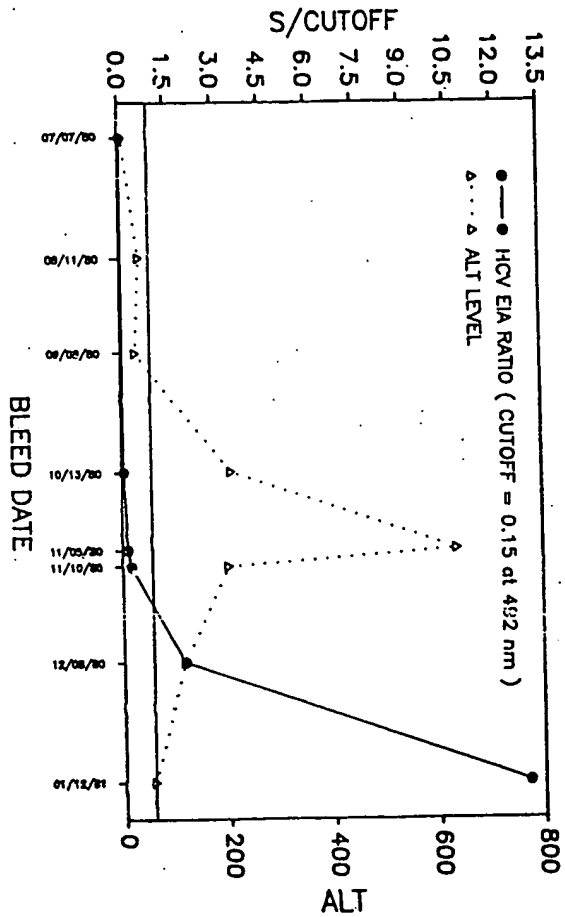
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510133

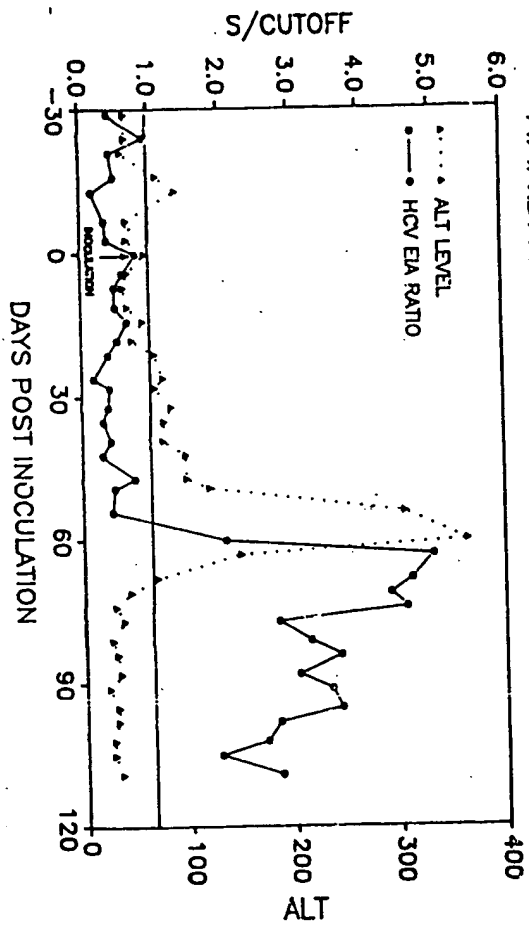
Fig 4-2

# SEROCONVERSION PANEL II DIALYSIS PATIENT WITH NANBH (NYBC)



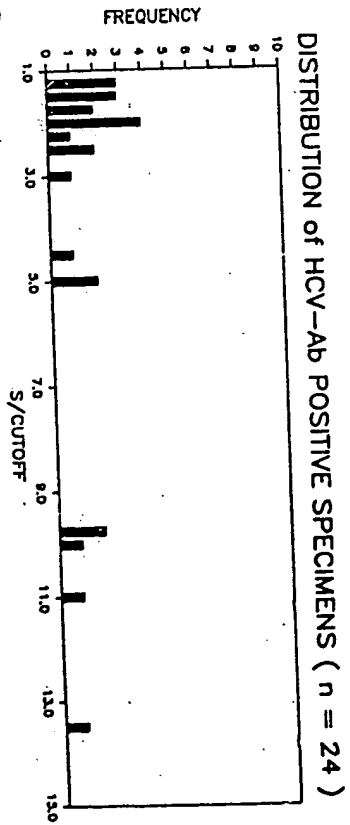
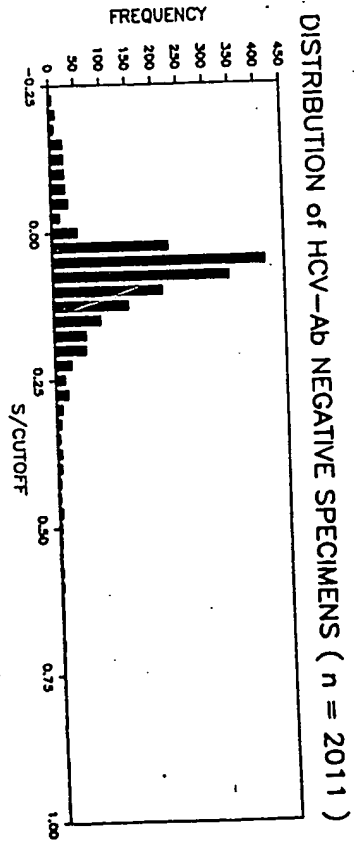


# SEROCONVERSION PANEL III NANBHV INOCULATED CHIMP (CDC)



11/28/510153  
8-1

8-2



01510153

108

# SYNTHETIC PEPTIDE BASED HCV NEUTRALIZATION EIA AS A CONFIRMATORY TEST

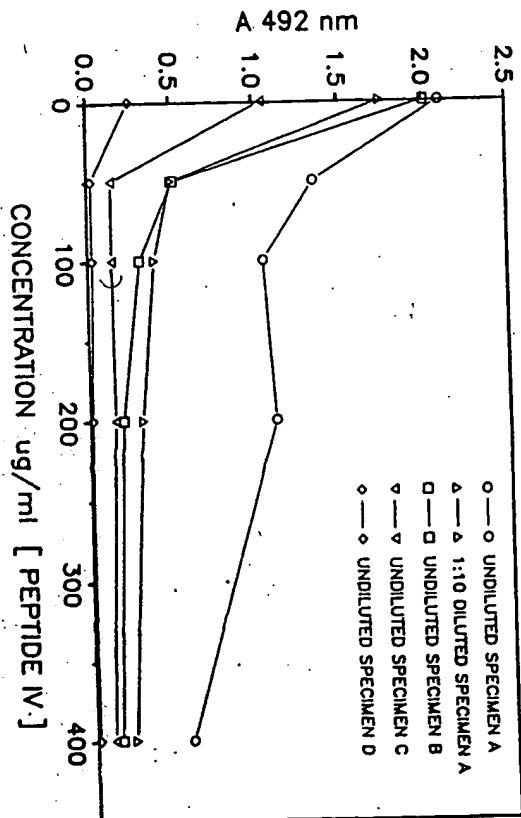
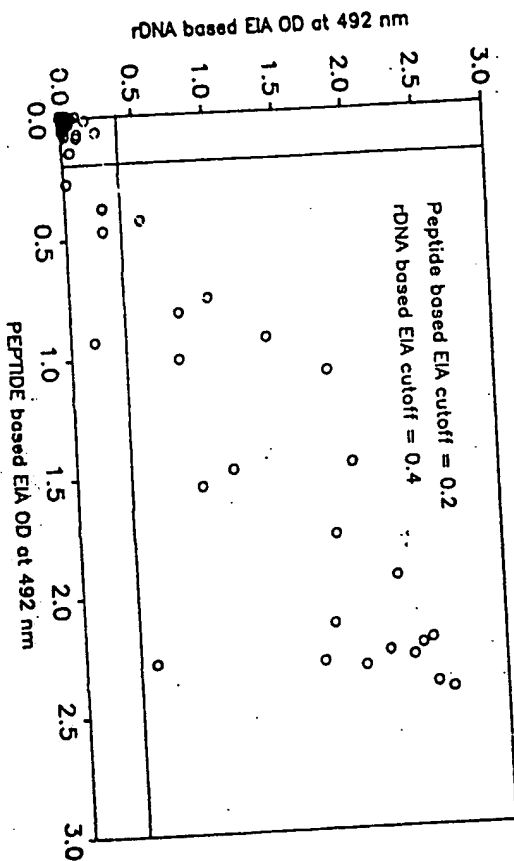


Fig 10

COMPARISON of PEPTIDE based and rDNA based HCV EIAs





**THOMSON**

---

FILE HISTORY

US 5,106,726

PATENT: 5,106,726

INVENTORS: Wang, Chang Y.

TITLE: Synthetic peptides specific for the  
detection of antibodies to HCV

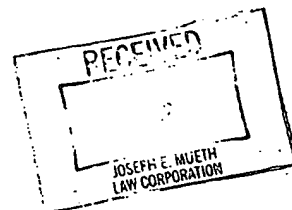
SERIAL NO: 558,799

FILED: 26 NUL 1990

ISSUED: 21 APR 1992

COMPILED: 29 DEC 2006

DOCKET: 323-100US



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SERIAL NUMBER	FILING DATE	CLASS	SUBCLASS	GROUP ART UNIT	EXAMINER
07/558,799	07/26/90	435 435	005	182 134 124	LEE

CHANG Y. WANG, GREAT NECK, NY.

**\*\*CONTINUING DATA\*\*\*\*\***  
 VERIFIED THIS APPLN IS A CIP OF 07/481,348 02/16/90 *Res-living*  
 AND A CIP OF 07/510,153 04/16/90 *ABN*  
 2.1

**\*\*FOREIGN/PCT APPLICATIONS\*\*\*\*\*** *None*  
 VERIFIED *file*

FOREIGN FILING LICENSE GRANTED 08/21/90

\*\*\*\*\* SMALL ENTITY \*\*\*\*\*

Foreign priority claimed	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no	AS FILED	STATE OR COUNTRY	SHEETS DRWGS.	TOTAL CLAIMS	INDER CLAIMS	FILING FEE RECEIVED	ATTORNEY'S DOCKET NO.
35 USC 119 conditions met	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no	→	NY	27	43	4	\$ 341.00	1151-4043
Verified and Acknowledged <i>Examiner's Initials</i>								

MORGAN & FINNEGAN  
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 NEW YORK, NY 10154

**CERTIFICATE**  
*Sept 20 1994*  
**OF CORRECTION**

**RECEIVED**  
 DEC 15 1994

**TITLE** SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV,  
 DIAGNOSIS OF HCV INFECTION AND PREVENTION THEREOF AS VACCINES

12. 11/4/91

U.S. DEPT. of COMM. Pat. & TM Office - PTO-436L (rev. 10-7)

**PARTS OF APPLICATION** *27 sheets formal drawings 9/30/91*  
**FILED SEPARATELY**

NOTICE OF ALLOWANCE MAILED		PREPARED FOR ISSUE		CLAIMS ALLOWED	
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Mailed

1. Application \_\_\_\_\_ papers.

2. Prior art 12-10-903. Prior art a w/ (2) attached 12-10-904. Ref Mus. 2-19-91/215. Cit (1) 5/28/916. Andt B 5/28/917. (4. Non 31100) 8/15/91 8/9/918. Andt C (WES) - Drawing 9/30/919. Andt D (WES) - Journal 10-15-91/10/9110. Andt E (WES) 10/31/9111. Exam's Andt F/F 11/6/9112. Andt G (R. 311) 11/26/91 u/2513. Entered 12-11-9114. Entered 1/2/92

PTO GRANT APR 21 1992

15. Request for C of G 2-25-93 2/1716. DIRECTOR'S REPORT 7/14/9417. Notif. to Reg. In Cert. of Conv. Aug 4/199418. Req for Cert. of Correction 8/29/94

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530	324 325 326 327 328-330 331-333	1/30/91	2.22
514	12-15 17		
435	334		
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above up to date		11/19/91	2.22

SEARCH NOTES		
Parent files	Date	Exmr.
SW 17510, 153		
6 p.d. date		

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INTERFERENCE SEARCHED			
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435	5,752	11/19/91	2.22
514	12-15		
530	334-338 350		

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# INDEX OF CLAIMS

Claim		Date	
Final	Original		
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SYMBOLS

- ..... Rejected
- ..... Allowed
- (Through numeral) Canceled
- ..... Restricted
- N ..... Non-elected
- I ..... Interference
- A ..... Appeal
- D ..... Objected

Claim		Date	
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U.S. GOVERNMENT PRINTING OFFICE: 1990-256-989

PATENT NUMBER <i>7,921,435</i>		ORIGINAL CLASSIFICATION CLASS <i>435</i> SUBCLASS <i>005</i>																					
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APPLICANT'S NAME (PLEASE PRINT) <i>Wang</i>		<table border="1"> <thead> <tr> <th>CLASS</th> <th colspan="3">SUBCLASS (ONE SUBCLASS PER BLO)</th> </tr> </thead> <tbody> <tr> <td><i>435</i></td> <td><i>7.92</i></td> <td></td> <td></td> </tr> <tr> <td><i>514</i></td> <td><i>12</i></td> <td><i>13</i></td> <td><i>14</i></td> </tr> <tr> <td><i>530</i></td> <td><i>324</i></td> <td><i>325</i></td> <td><i>326</i></td> </tr> <tr> <td><i>530</i></td> <td><i>328</i></td> <td><i>329</i></td> <td><i>33</i></td> </tr> </tbody> </table>		CLASS	SUBCLASS (ONE SUBCLASS PER BLO)			<i>435</i>	<i>7.92</i>			<i>514</i>	<i>12</i>	<i>13</i>	<i>14</i>	<i>530</i>	<i>324</i>	<i>325</i>	<i>326</i>	<i>530</i>	<i>328</i>	<i>329</i>	<i>33</i>
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<i>G01N</i>	<i>33/53</i>																						
GROUP ART UNIT <i>1811</i>		ASSISTANT EXAMINER (PLEASE STAMP OR PRINT)  PRIMARY EXAMINER (PLEASE STAMP OR PRINT FULL NAME) <i>Lester L. LEE</i>																					

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## **File History Report - References**

**Other Publications** -- While copying the 'Other Publications' section of your file history we noticed that one or more pages and/or publications are missing from this portion of the file wrapper contents. Please note that all references have been copied as they appear in the file history. At your request, we will attempt to obtain the missing publication(s) from alternative resources. Please note that additional charges will apply to this service.

United States Patent [19]  
Wang

[11] Patent Number: 5,106,726  
[45] Date of Patent: Apr. 21, 1992

[54] SYNTHETIC PEPTIDES SPECIFIC FOR THE  
DETECTION OF ANTIBODIES TO HCV

[75] Inventor: Chang Y. Wang, Great Neck, N.Y.

[73] Assignee: United Biomedical, Inc., Lake  
Success, N.Y.

[21] Appl. No.: 558,799

[22] Filed: Jul. 26, 1990

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 481,348, Feb. 16, 1990,  
abandoned, and a continuation-in-part of Ser. No.  
510,153, Apr. 16, 1990, abandoned.

[51] Int. Cl.<sup>3</sup> ..... C12Q 1/70; C07K 7/08;  
C07K 7/10; G01N 33/53

[52] U.S. Cl. .... 435/5; 435/7.92;  
514/12; 514/13; 514/14; 514/15; 530/324;  
530/325; 530/326; 530/327; 530/328; 530/329;  
530/330

[58] Field of Search ..... 530/324, 325, 326, 327,  
530/328-330, 350; 514/12-15; 435/7.92, 5

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Primary Examiner—Lester L. Lee  
Attorney, Agent, or Firm—Maria C. H. Lin

[57] ABSTRACT

The present invention relates to a method for the detection in body fluids of antibodies to hepatitis C virus (HCV), also known as a non-A non-B hepatitis (NANBH) virus and to the diagnosis of NANBH by the use of a composition of synthetic peptides. Each of these peptides has an amino acid sequence corresponding to immunodominant regions of a fusion protein and a non-structural polypeptide of HCV, SOD/HCV C100 and a postulated HCV structural (core) protein. More specifically, the present invention is directed to the use of a group of synthetic peptides in a prescribed sequence or their analogues for the detection of antibodies to HCV in body fluids. The detection method includes an enzyme-linked immunosorbent assay (ELISA), and other forms of immunoassay procedures.

The present invention also relates to a method for generating high titer antibodies to HCV in healthy mammals, including humans, by the use of compositions containing these synthetic peptides, analogues or mixtures thereof, in a free, conjugated or polymeric form as key components in synthetic vaccines for the prevention of non-A non-B hepatitis (NANBH).

37 Claims, 27 Drawing Sheets

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FIG. 1-1.

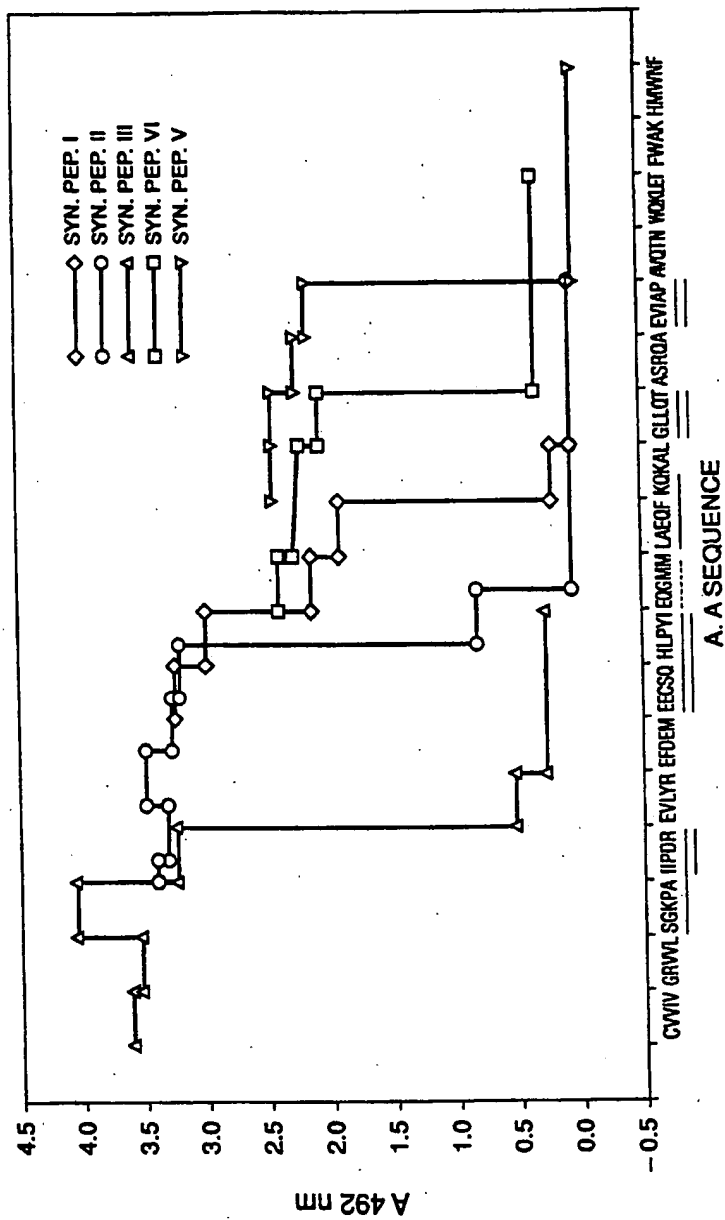


FIG. 1-2.

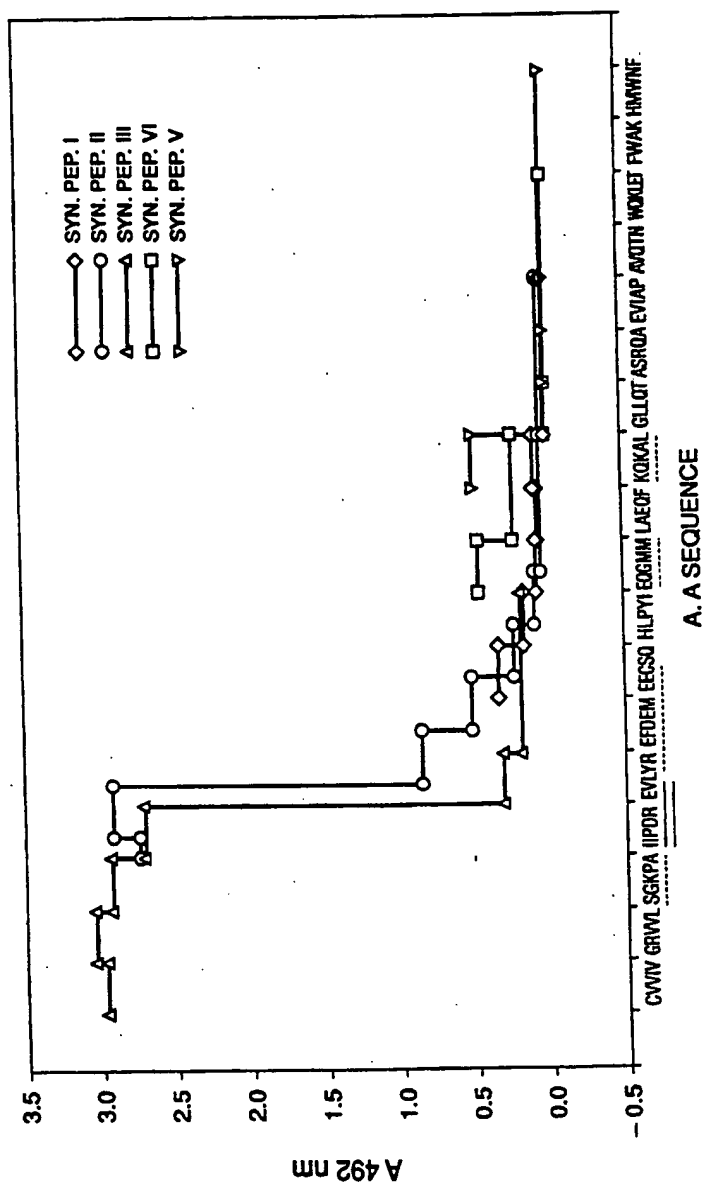




FIG. 1-3.

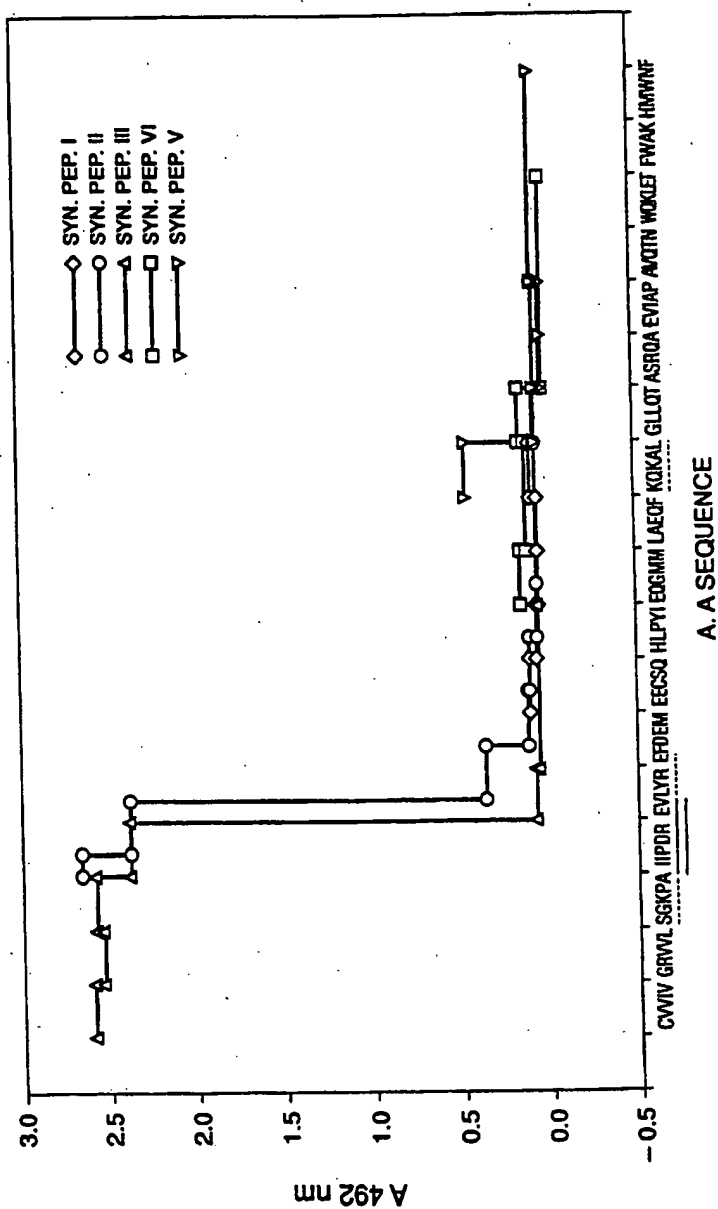


FIG. 1-4.

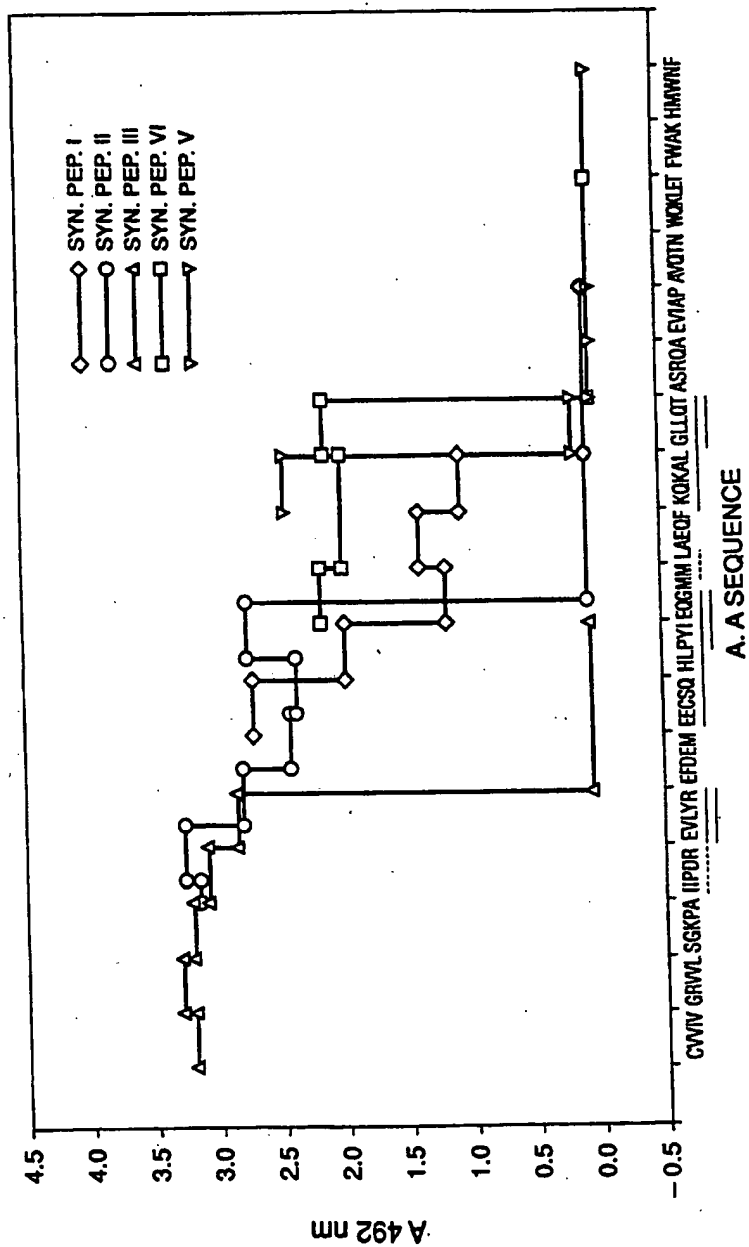


FIG. 2-1.

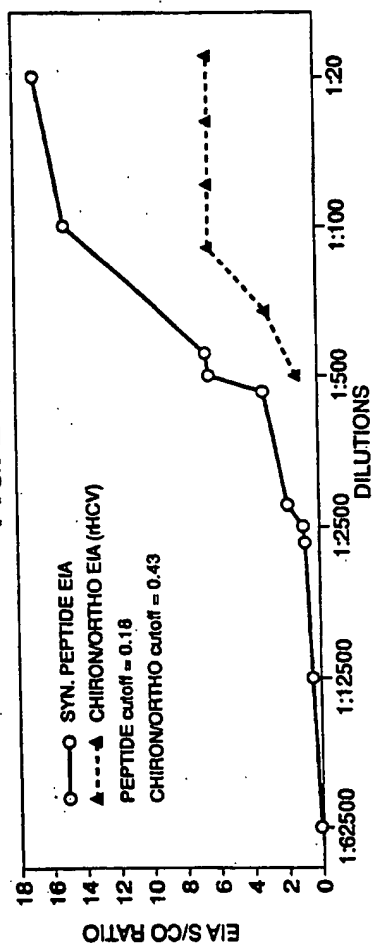
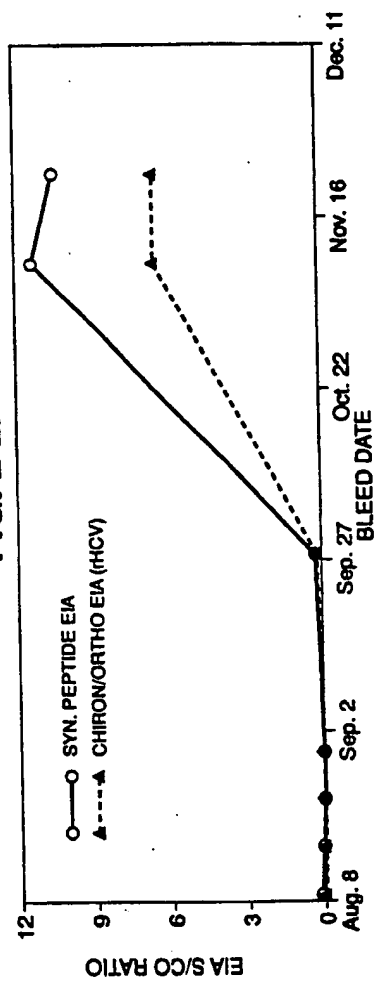


FIG. 2-2.



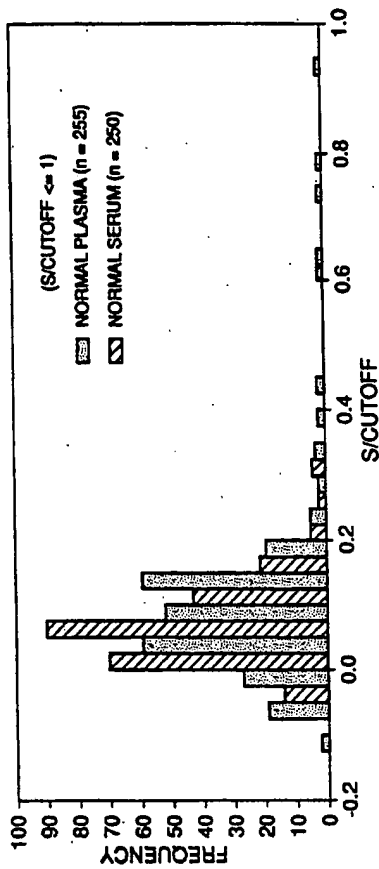


FIG. 3-1.

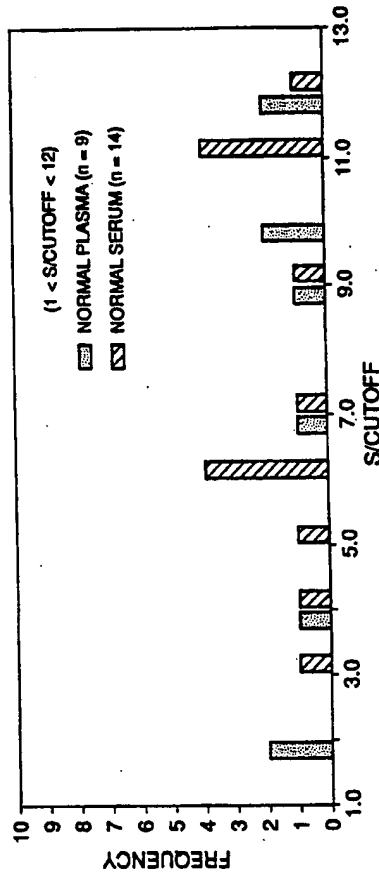


FIG. 3-2.

FIG. 4.

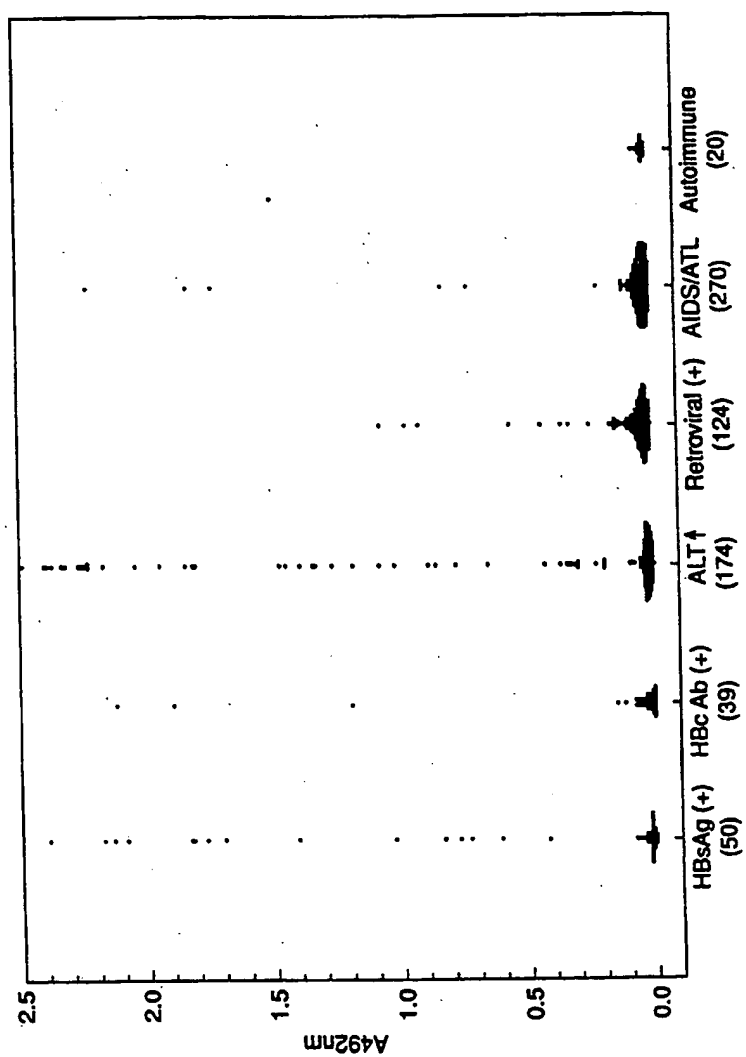


FIG. 5.

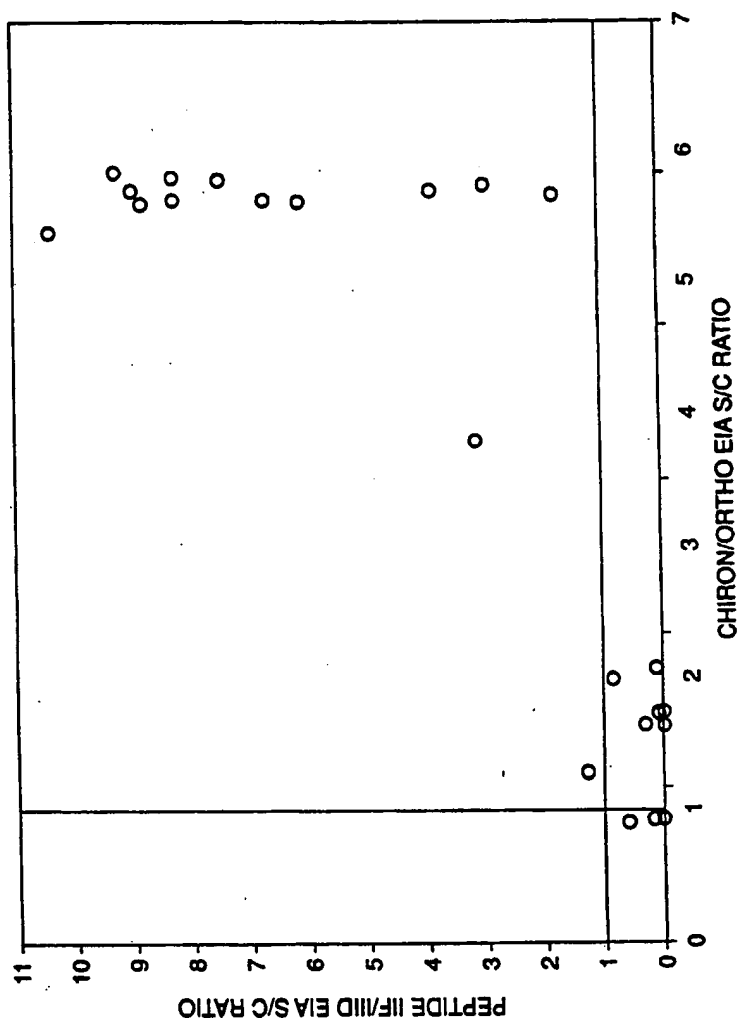


FIG. 6.

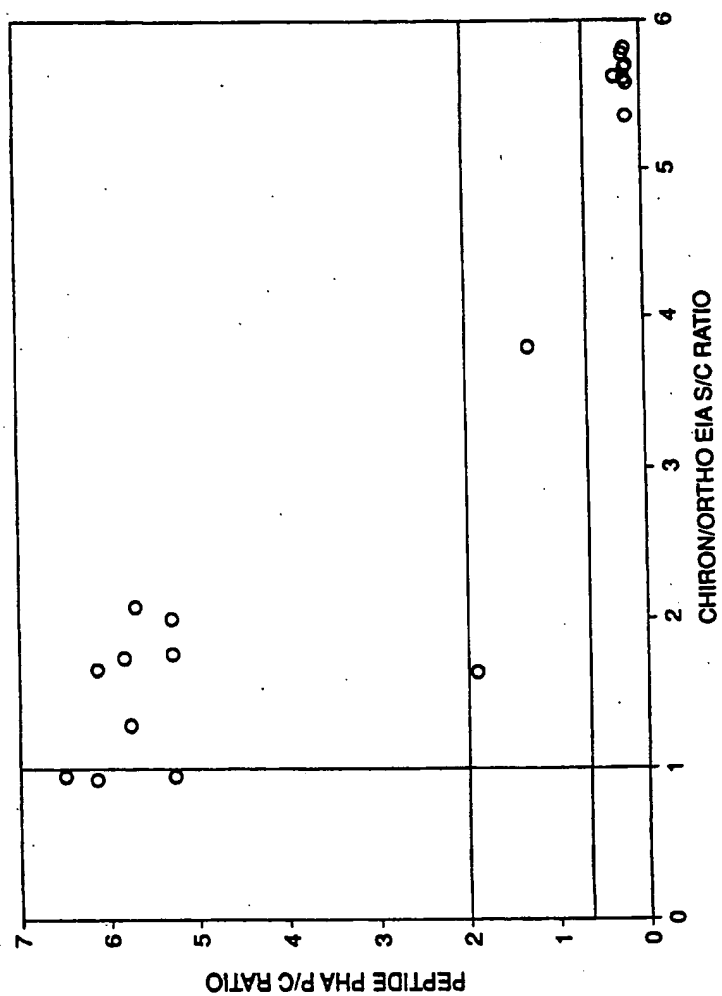


FIG. 7-1.

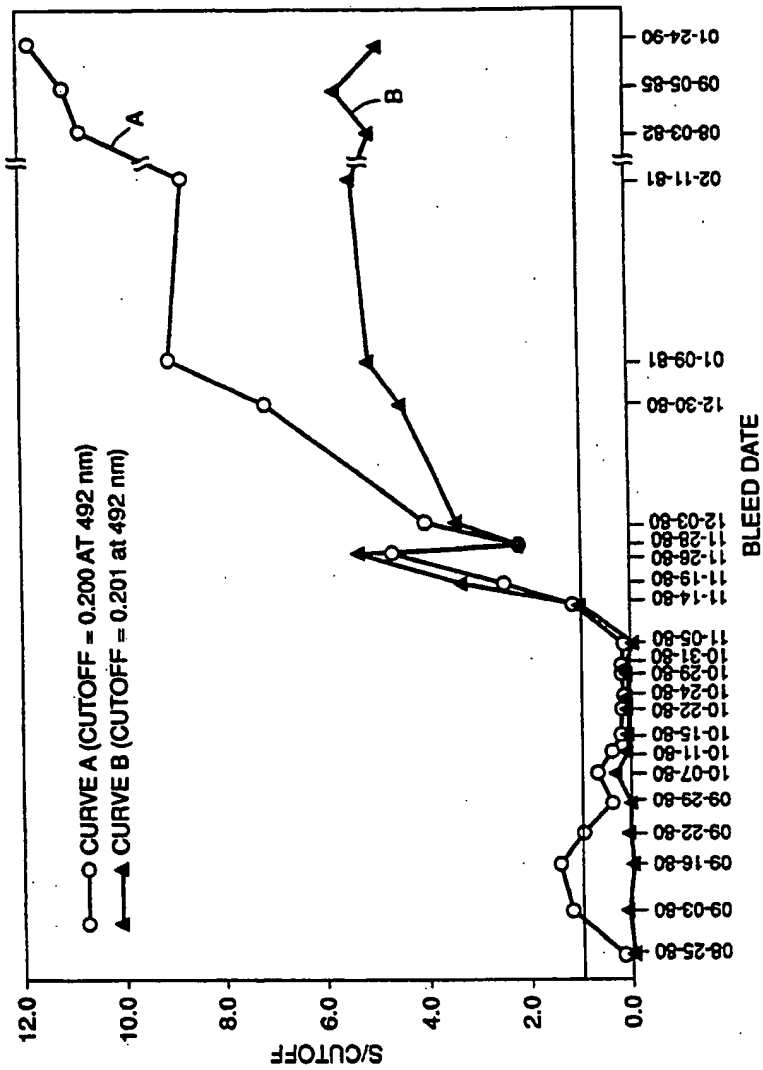




FIG. 7-2.

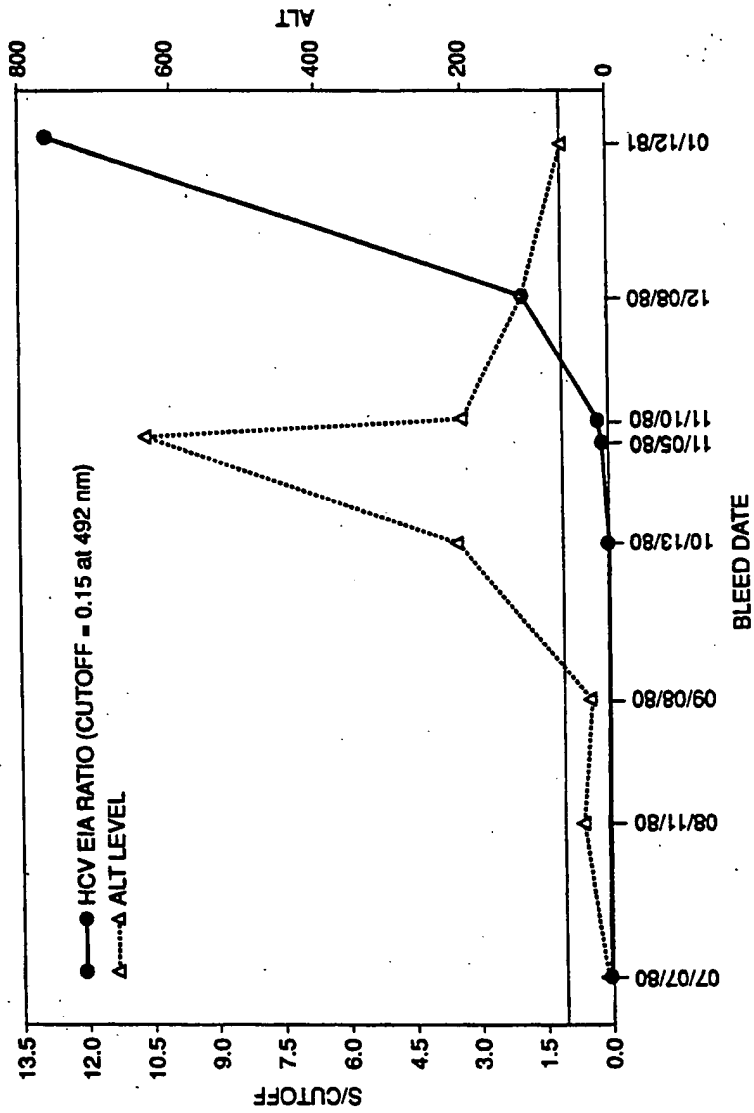
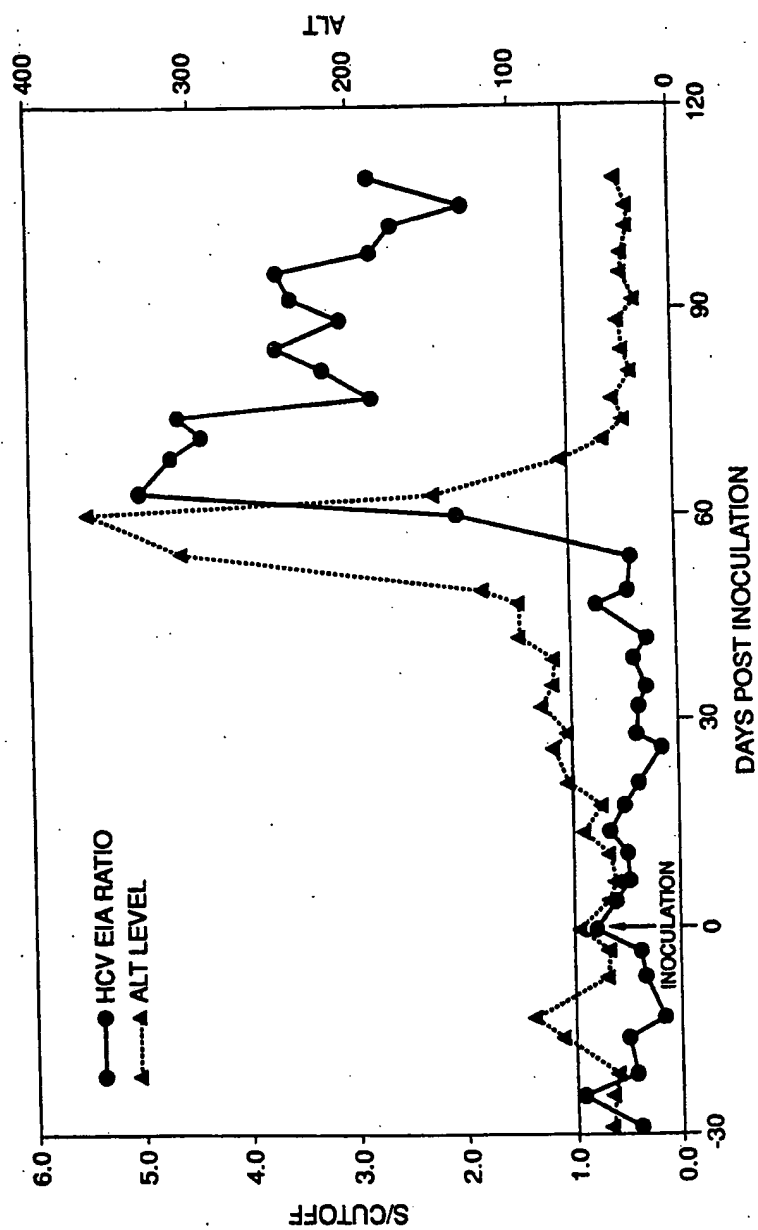


FIG. 7-3.



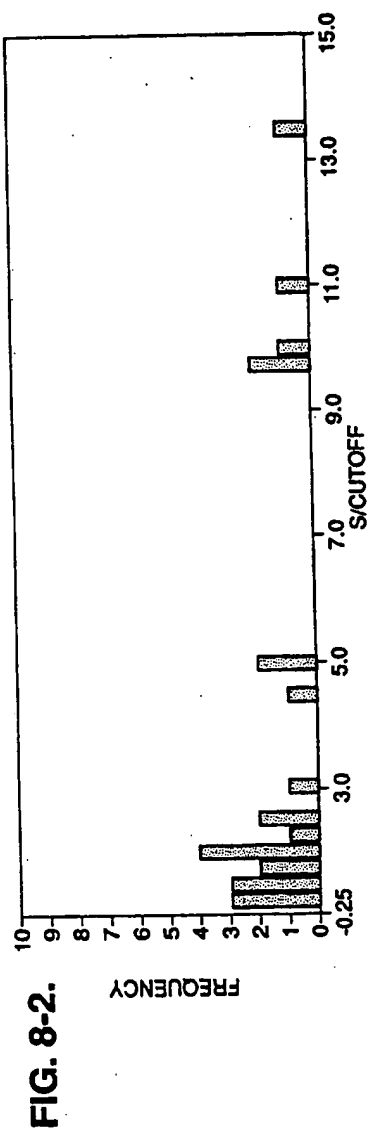
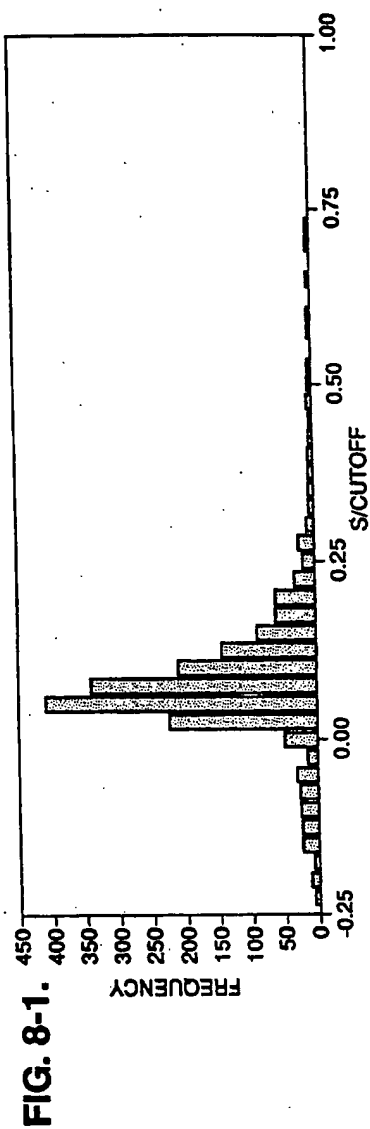


FIG. 9.

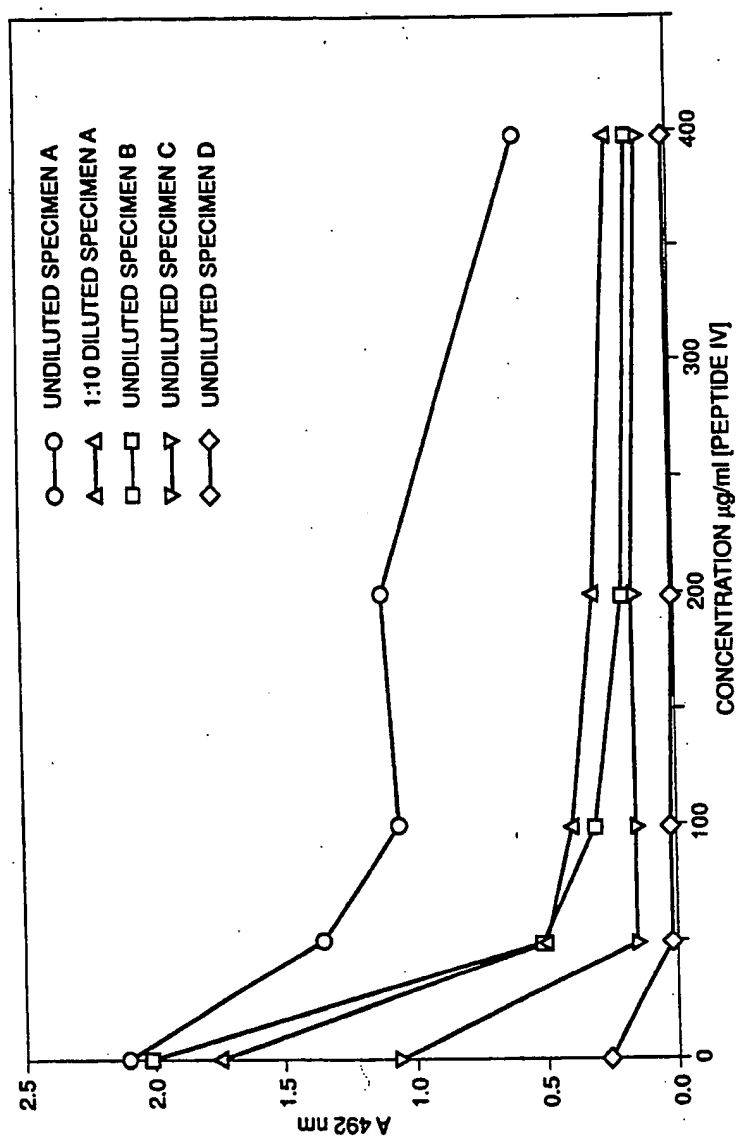


FIG. 10.

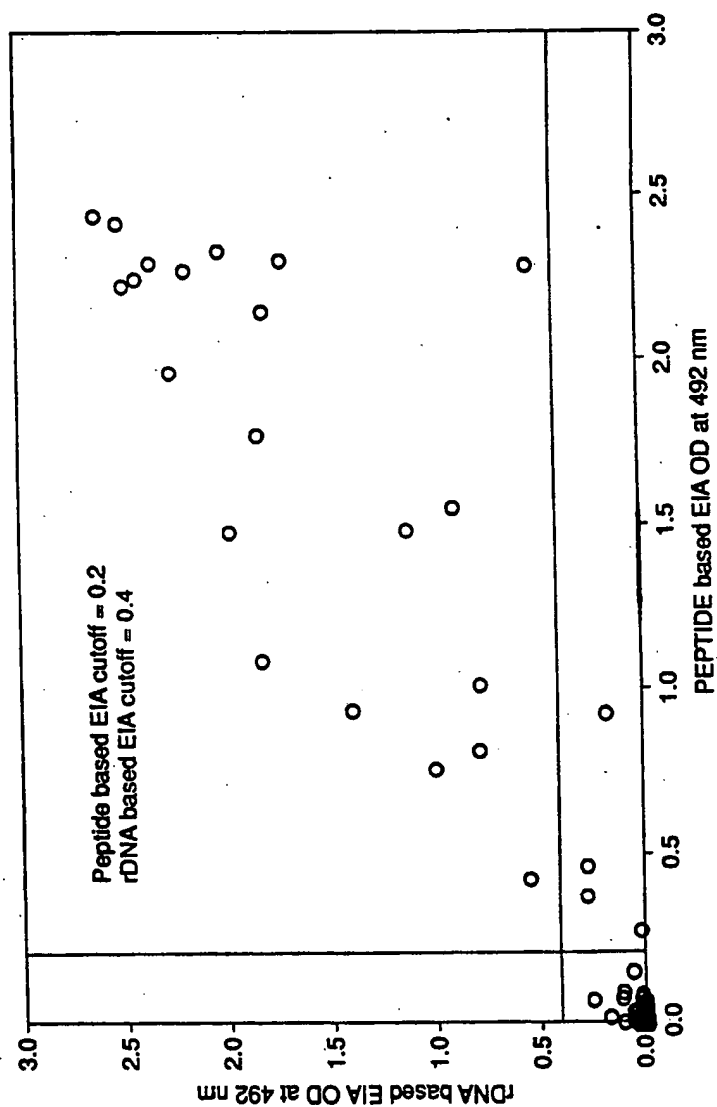


FIG. 11-1.

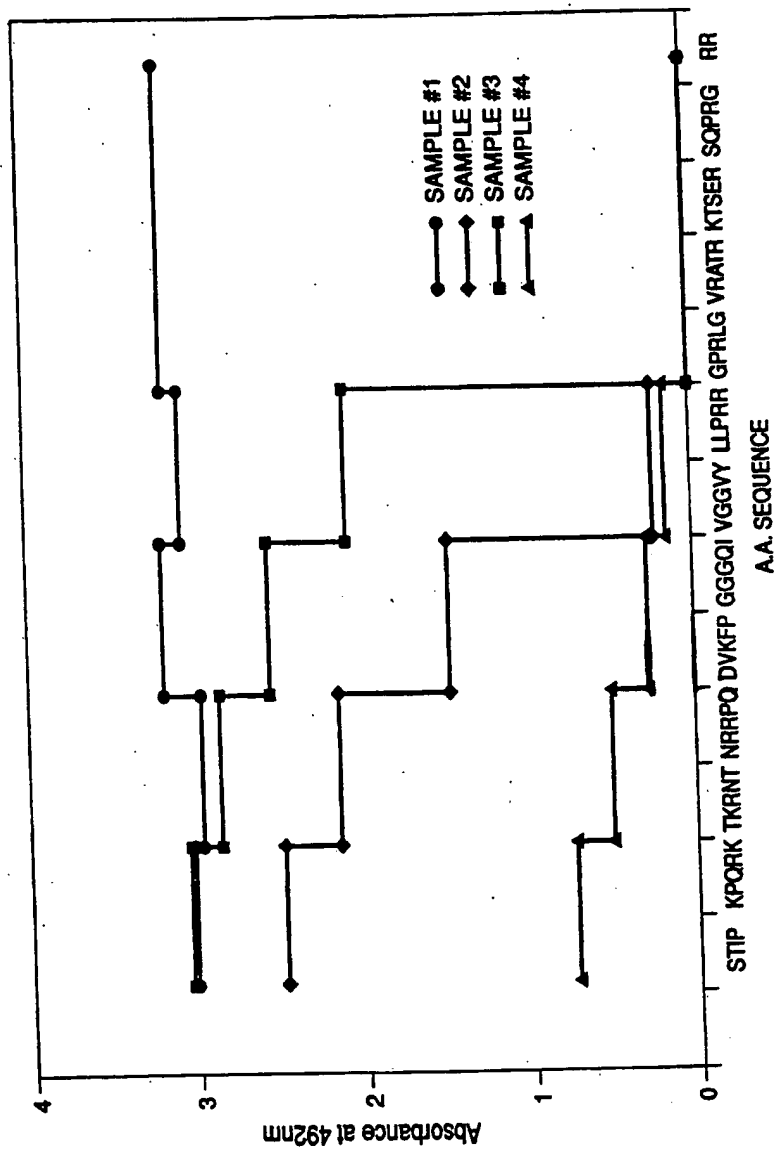


FIG. 11-2.

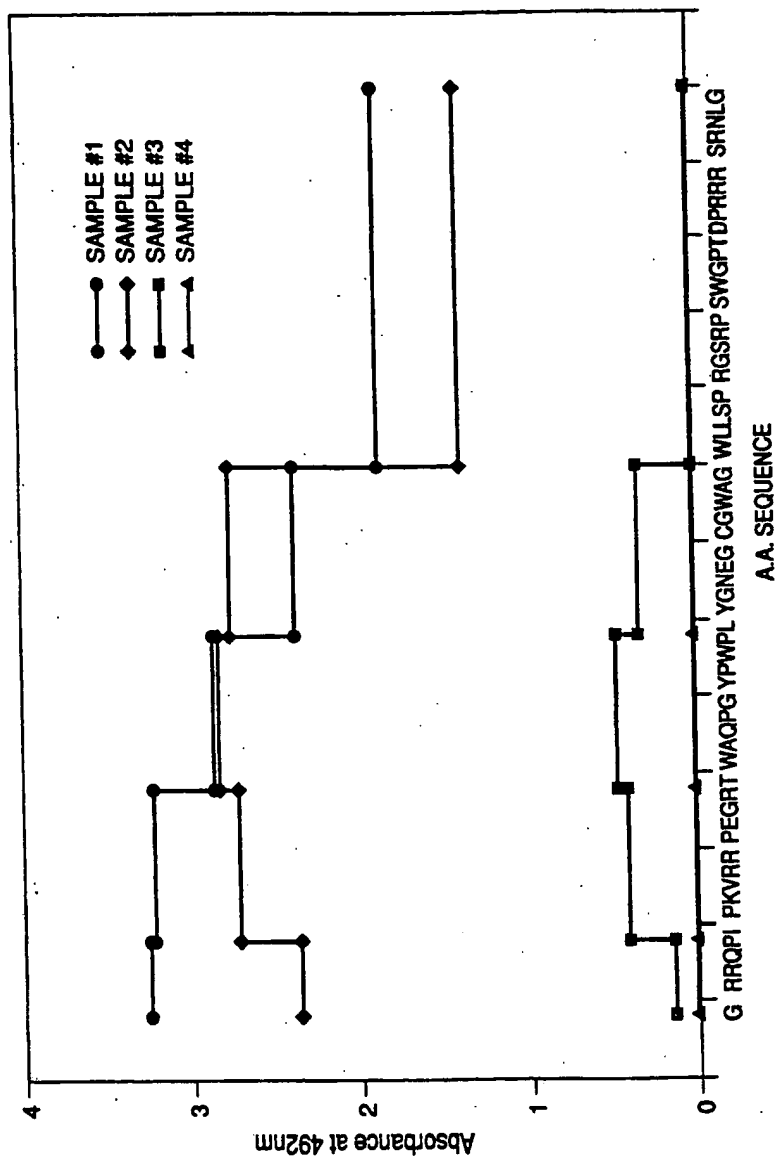


FIG. 12-1.

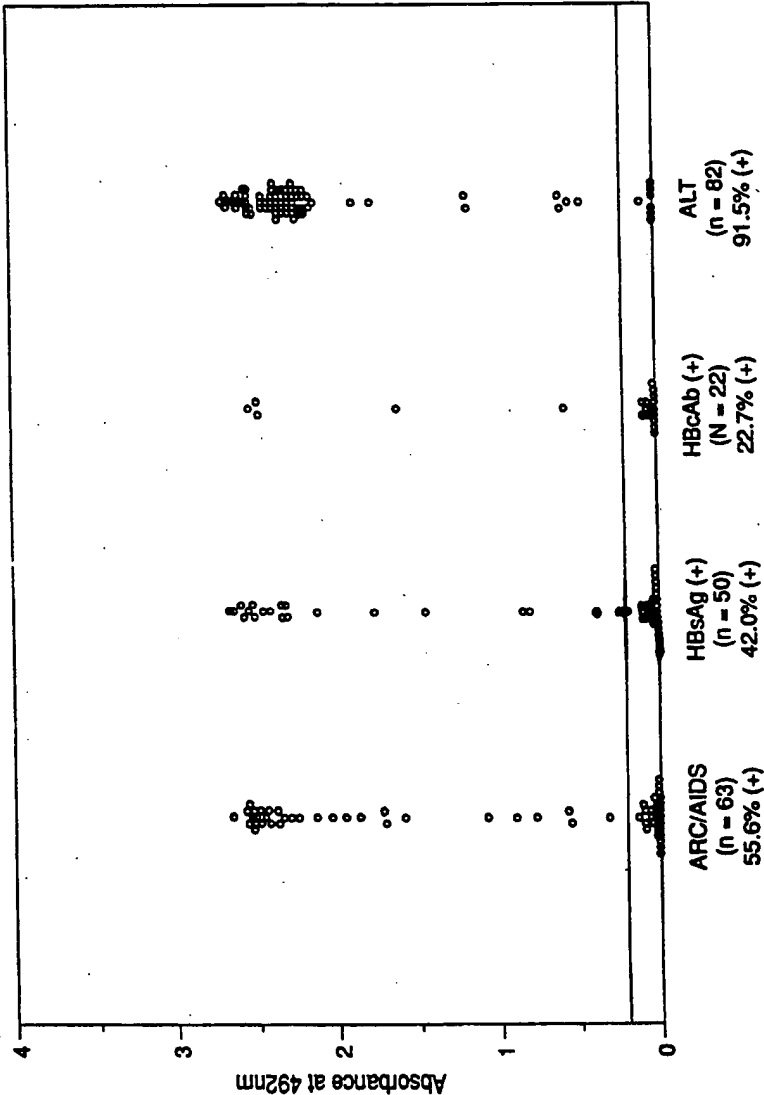




FIG. 12-2.

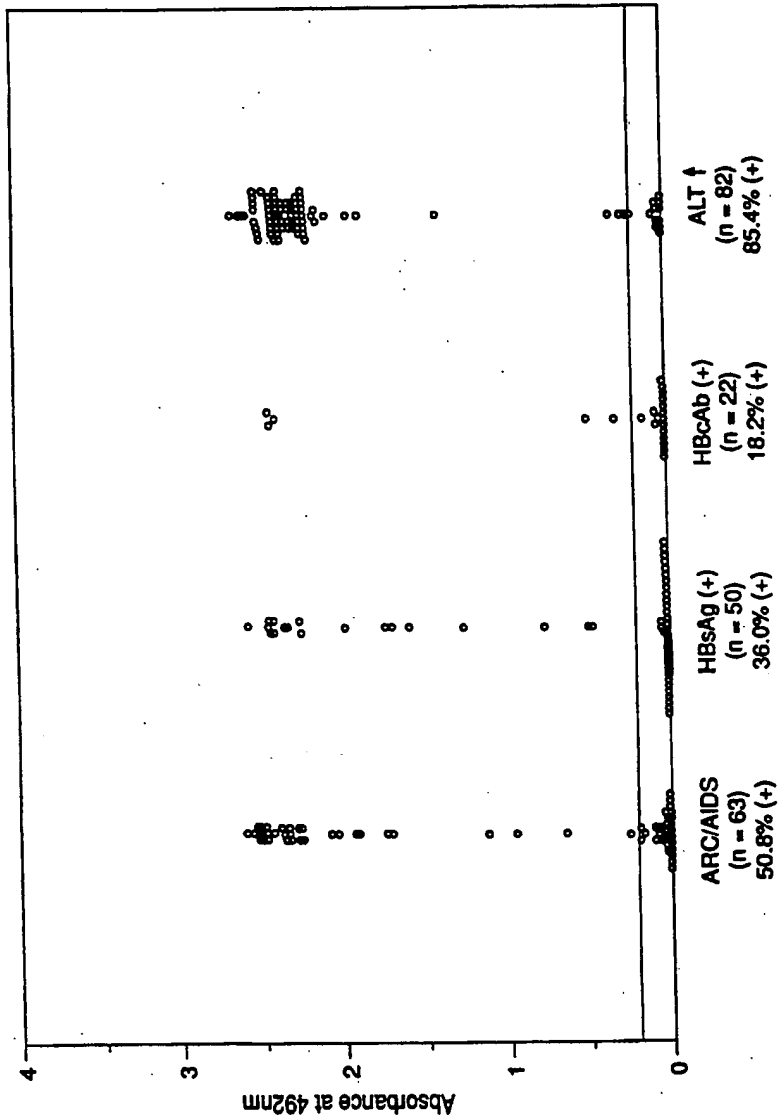
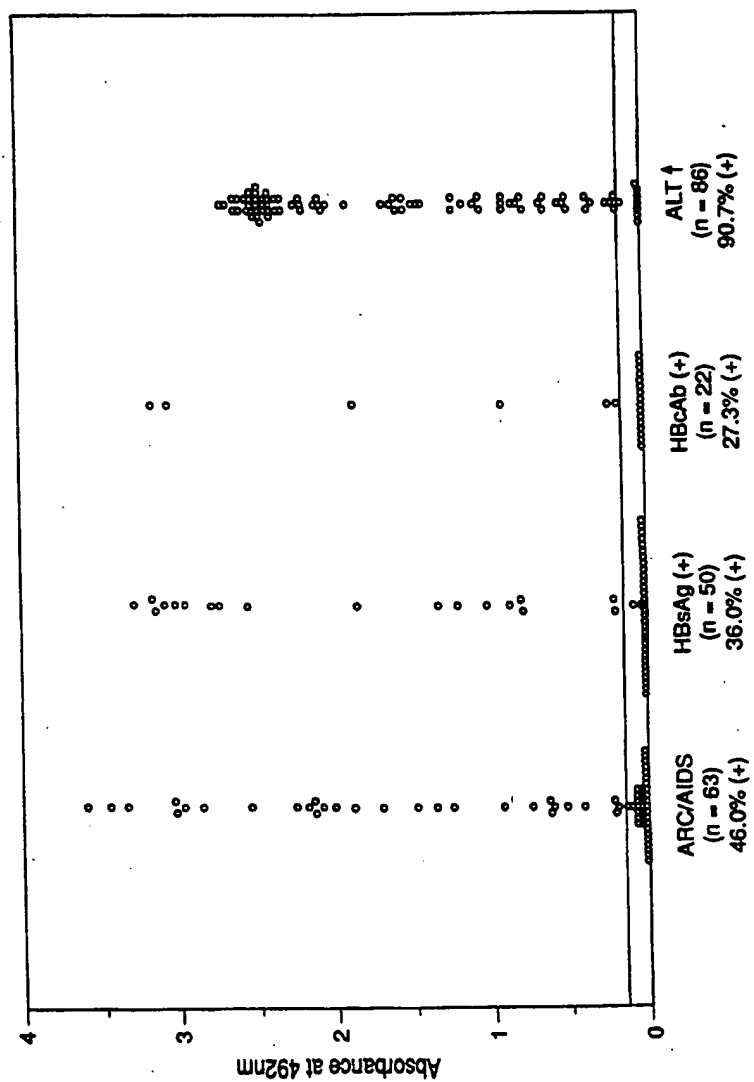


FIG. 12-3.



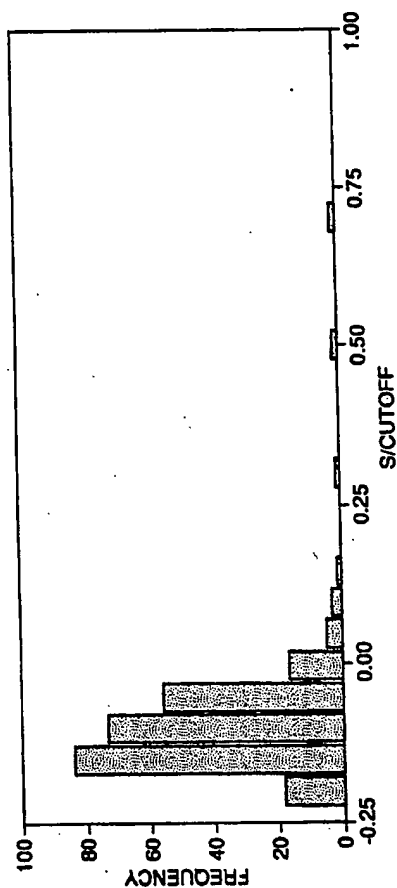


FIG. 13-1.

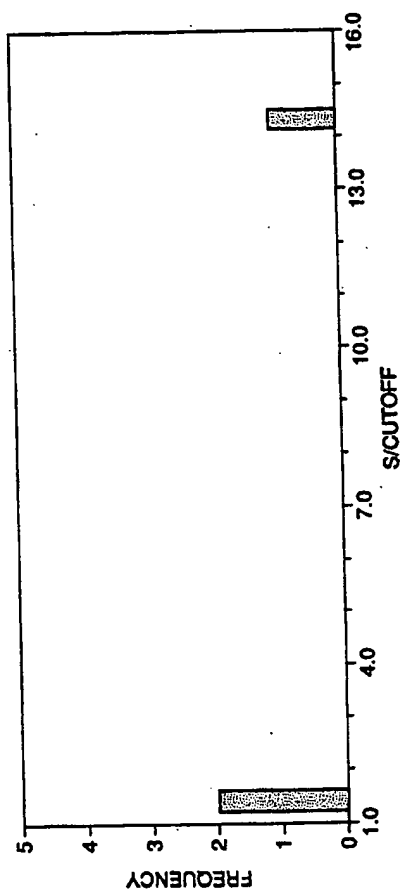


FIG. 13-2.

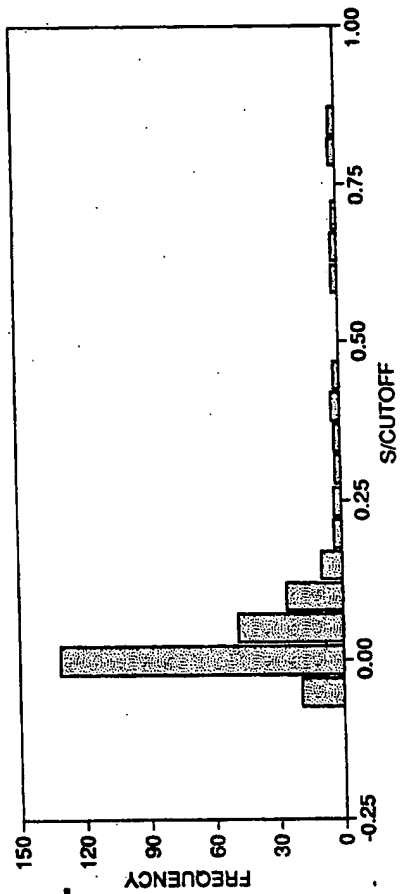


FIG. 13-3.

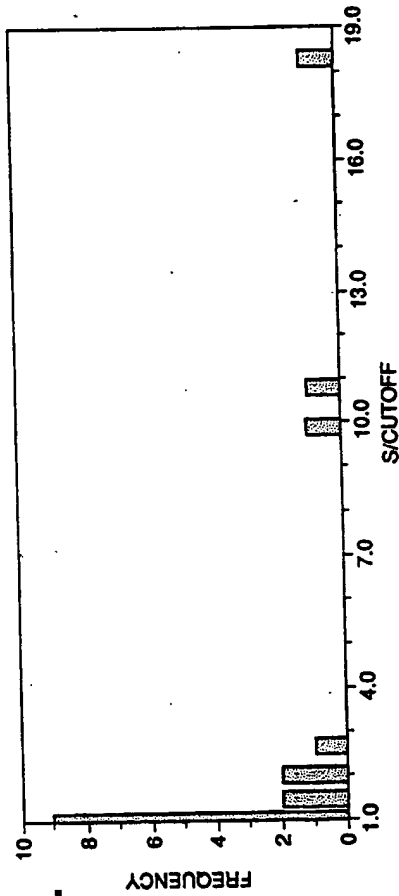


FIG. 13-4.

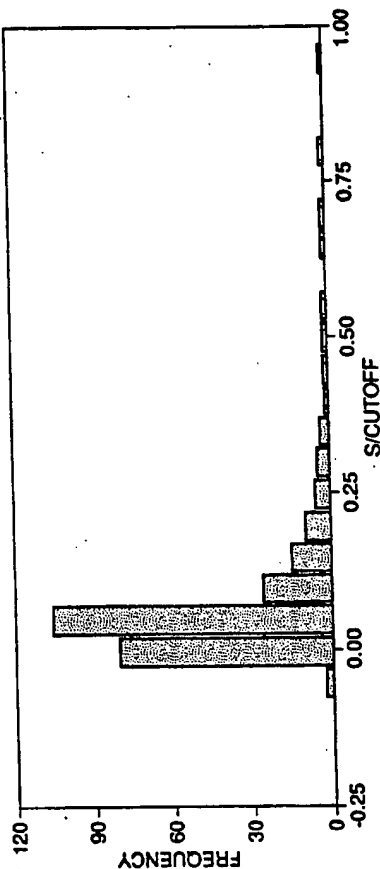


FIG. 13-5.

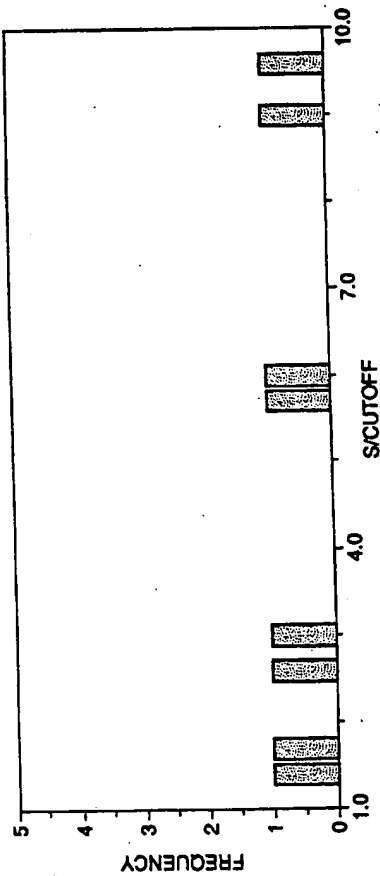


FIG. 13-6.

FIG. 14-1.

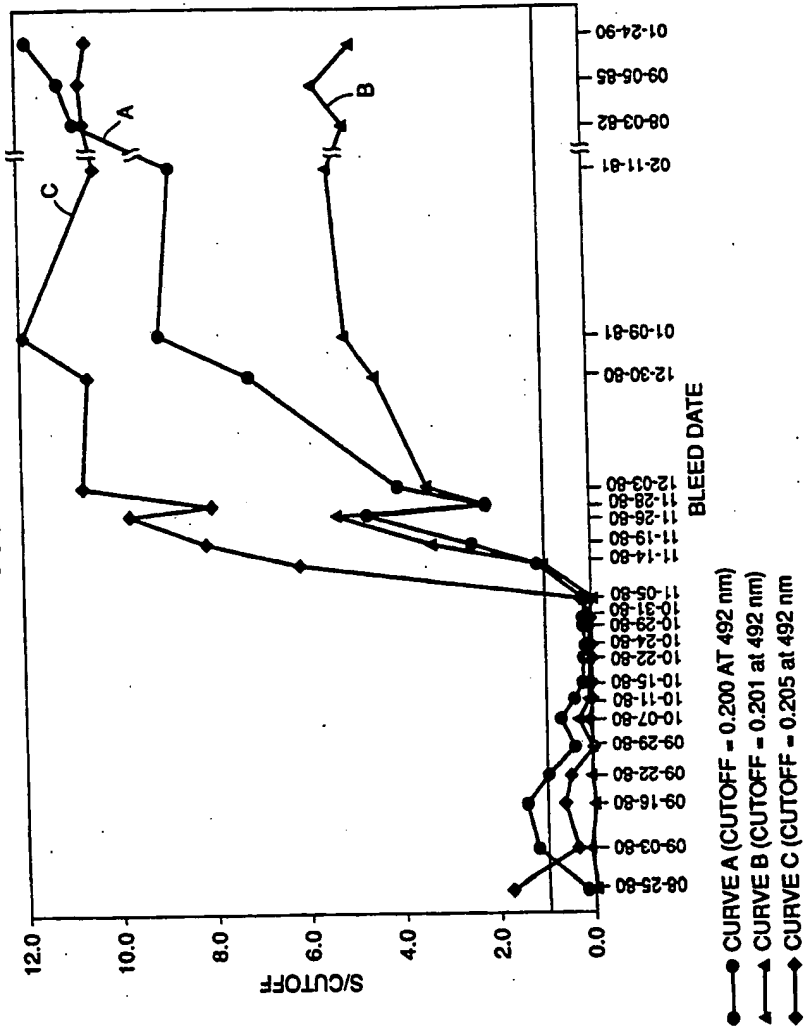
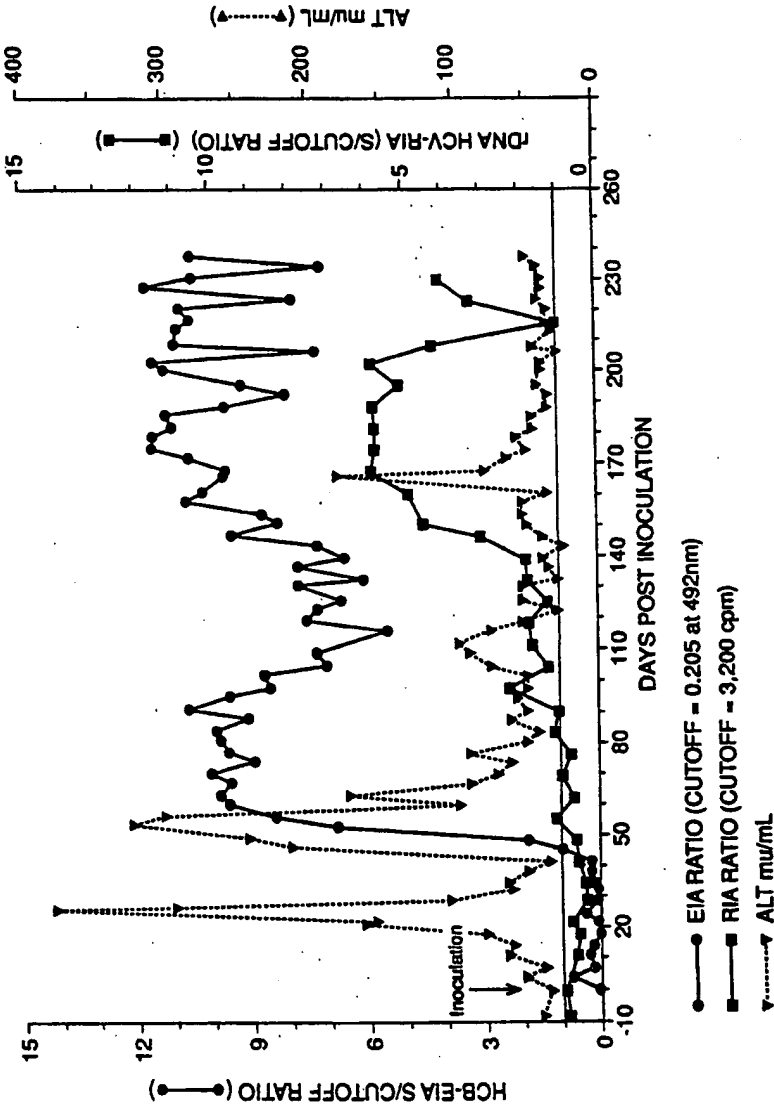
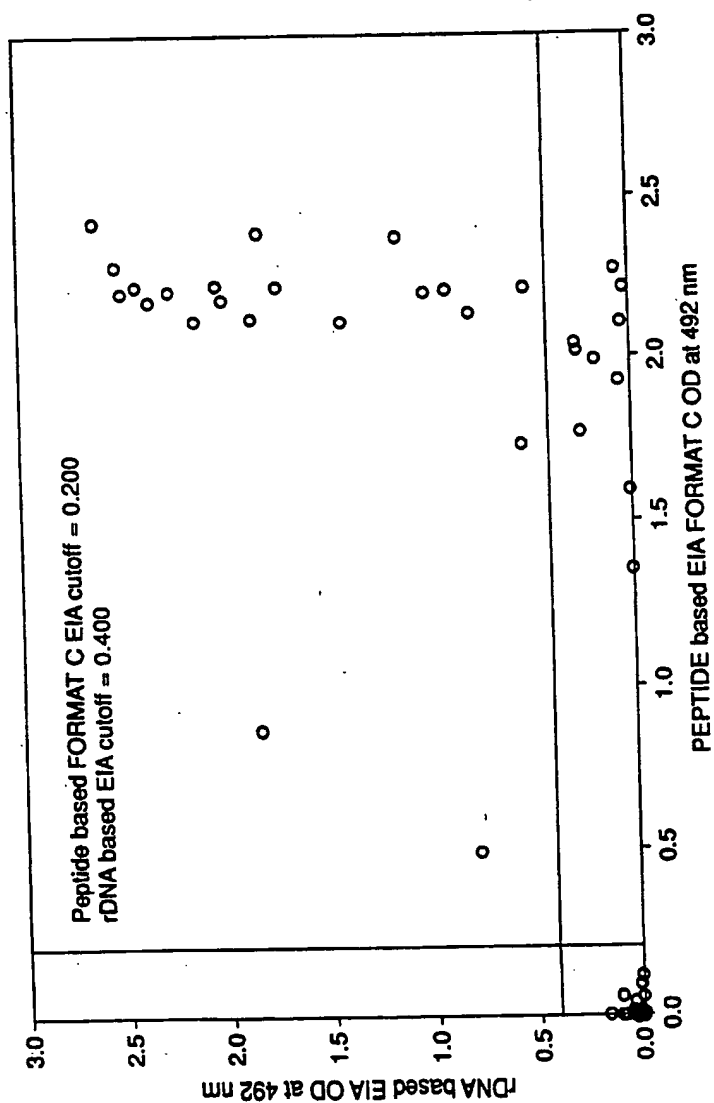


FIG. 14-2.



**FIG. 15-1.**





Peptide based EIA FORMAT A OD at 492 nm

Peptide based EIA FORMAT C OD at 492 nm

Peptide based FORMAT A EIA cutoff = 0.252

Peptide based FORMAT C EIA cutoff = 0.200

# SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV

## CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation in part application of copending application Ser. No. 07/481,348 and now abandoned, filed Feb. 16, 1990, and application Ser. No. 07/510,153 and now abandoned, filed Apr. 16, 1990.

## INTRODUCTION

The present invention relates to peptide compositions specific for the diagnosis and prevention of hepatitis C virus (HCV) infection, or non-A non-B hepatitis (NANBH). More particularly, the present invention is directed to synthetic peptide compositions which are specific for the detection of antibodies to HCV in body fluids and immunoassays using the same. The invention also includes the use of the synthetic peptide compositions as antigens for eliciting the production of monoclonal and polyclonal antibodies against HCV and as immunogens in vaccines for the prevention of NANBH or HCV infection.

In the 1940's, two independent investigators concluded that there were at least two types of viral hepatitis, designated as A and B (HAV and HBV) and that infection by one type, either HAV or HBV, did not confer the patient with cross-immunity (1-3). It was only in the 1970's with the introduction of serologic markers for hepatitis A and hepatitis B that it became possible to identify diseases caused by the two viruses and to distinguish between these two types of hepatitis clinically and serologically.

Subsequently, in 1974, Prince et al. suggested that many cases of transfusion hepatitis could not be attributed to HAV or HBV and were caused by an agent other than these viruses. They proposed naming the agent hepatitis C virus (HCV) (4). The presence of another hepatitis causing agent was subsequently confirmed by Alter et al., who reported that although the exclusion of commercial blood donors found to carry hepatitis B surface antigen (HBsAg) significantly reduced the frequency of post-transfusion hepatitis (5), 7 to 10 percent of the 5 million Americans who received transfusions each year still developed hepatitis. In 90% of these post-transfusion hepatitis cases, a specific virus, unrelated to HAV, HBV, Epstein-Barr virus, cytomegalovirus or other viruses which occasionally produce liver diseases, was implicated as the etiologic agent (5). This infection was designated as non-A non-B hepatitis (NANBH).

Over the years, NANBH has been reported in patients undergoing hemodialysis, recipients of renal transplants (6), intravenous drug abusers (7) and patients in institutions for the mentally retarded (8). Further, nurses caring for patients with NANBH have also been found to contract this disease.

Epidemiologic evidence suggests that there may be three types of NANBH: the water borne epidemic type; the blood or needle associated type; and the sporadically occurring community acquired type. However, the number and precise nature of the causative agents of NANBH still remain not entirely clear.

The acute phase of NANBH is less severe than that of hepatitis B, and the disease is rarely fatal. However, more than a third of the individuals who contract NANBH develop a chronic form of the disease in

which they may remain infectious indefinitely. This chronic state may lead to cirrhosis of the liver and eventually to liver cancer.

Many methods have been developed in an attempt to detect the putative NANBH viral antigens and antibodies. These include agar-gel diffusion, counter immunoelectrophoresis, immunofluorescence microscopy, immunoelectron microscopy, radioimmunoassay, and enzyme-linked immunosorbent assay using crude biologic lysates and antibodies from patients. However, none of these assays are sufficiently sensitive, specific, and reproducible for use as a diagnostic test for NANBH. Some of the reactivities detected were later attributable to the presence of antibodies to host cytoplasmic antigens or low levels of a rheumatoid-factor-like substance frequently present in patients with or without hepatic diseases.

In the absence of a definitive test for NANBH, the diagnosis in the past has been one of exclusion. It was based on the clinical presence of acute hepatitis and the persistent absence of serologic markers for hepatitis A and B, Epstein-Barr virus or cytomegalovirus.

Because no specific test for the detection of antibodies to NANBH or HCV has been available, the use of nonspecific tests to screen donors has been adopted in the past decade as a means of preventing at least some post-transfusion NANBH.

One such surrogate test measures liver enzyme levels. The concentrations of some of the liver enzymes, in particular alanine aminotransferase (ALT), are frequently elevated in the blood of patients with active hepatitis. Two independent studies have shown a correlation between donor ALT levels and the incidence of NANBH in transfusion recipients (9-11). However, some studies showed that only about 20 percent of blood donors who transmitted NANBH have elevated liver enzyme concentrations. Other investigators, furthermore, have found that the liver enzyme levels can be increased by extraneous factors, such as heavy drinking.

Epidemiologic circumstances predisposing donor populations to infection with hepatitis B virus may also favor exposure to NANBH agents. A study conducted by Stevens et al. (12) evaluated the risk factors in donors for the presence of antibodies to hepatitis B virus. The results indicated that units of blood which were positive for antibodies to the hepatitis B core antigen (anti-HBc) appeared to present a two to three-fold greater risk of NANBH in the recipients than units without anti-HBc. They concluded that anti-HBc screening of donors might prevent about one third of the cases of NANBH attributable to transfusion, whereas ALT screening might prevent nearly one half of the cases of post transfusion NANBH.

Even with the use of these surrogate tests to establish the diagnosis of NANBH by exclusion, the correct identification of the NANBH carriers was still far from satisfactory. Firstly, there are a significant number of patients who received blood lacking the surrogate markers and yet developed NANBH. Secondly, there is a minimal overlap between donors with elevated ALT levels and those with anti-HBc. Lastly, there are recipients of blood units which were positive for a surrogate marker, but who did not become infected with NANBH, also known as HCV (13-15).

Thus, there is an urgent demand for a sensitive and specific method to identify carriers of NANBH and to

screen out contaminated blood or blood products. In addition, there is also a need for an effective vaccine and/or therapeutic agent for the prevention and/or treatment of the disease.

Recently, a group of scientists at Chiron Corp. constructed a random-primed complementary DNA (cDNA) library from plasma containing the uncharacterized NANBH agent (16). They screened the library with serum from a patient diagnosed with NANBH and isolated a cDNA clone that encodes an antigen associated specifically with NANBH. This clone was found to be derived from the genome of an agent similar to the togaviridae or flaviviridae (16). The newly identified NANBH agent was called hepatitis C virus (HCV). A specific assay for this blood-borne NANBH virus was developed based on a fusion polypeptide of human superoxide dismutase (SOD) and 363 HCV amino acids, designated as SOD/HCV C100-3 (17). SOD/HCV C-100-3 was cultured from a clone of recombinant yeast, purified, and used to capture circulating viral antibodies (17). A family of cDNA sequences derived from this hepatitis C virus was subsequently reported in detail (18).

However, the nucleotide sequence of HCV disclosed by the Chiron group covers only about 75% of the HCV genome and represents only the nonstructural genes.

More recently Mayumi, et al. determined the 5'-terminal sequence of the genome of HCV for two distinct HCV strains in human and chimpanzee carriers (27).

The 5'-terminal sequence contained a 5' non-coding region of at least 324 nucleotides, well preserved in the two strains. The non-coding region was followed by a coding region of 1348 nucleotides continuing beyond the reported sequence of the prototype HCV which spanned 7310 nucleotides (18). Based on these results (18,27), HCV is considered to possess an uninterrupted open reading frame encoding at least 2886 amino acid residues.

A comparison of the complete nucleotide sequence of the Hepatitis C virus to that of other Flaviviruses (28) has led us to postulate that two structural genes encoding for the core (or nucleocapsid protein) and the envelope proteins were contained in the HCV genome located in the upstream and downstream region respectively of the 5'-terminal sequence as reported by the Mayumi group (27). By careful analysis of the whole HCV genome structure and the predicted amino acid sequence encoded in the structural and non-structural proteins, we have now identified and characterized by an extensive series of experiments and through serological validation, the immunodominant regions of the HCV proteins.

The predicted amino acid sequence of the HCV genome is presented in Table 6, wherein the sequence for (a) is the sequence for J-1 (27, 29), (b) is the sequence for J-4 (27) and (c) is the sequence for the prototype PT (18). These show where conservative substitutions, deletions or substitutions can be made.

TABLE 6

(a)	MSTI PKPQRKTKRNTNRRPQDVKEFGGGQI VGGVYLLPRRGPRLGVRATR	50
(b)	-----N-----	
(c)	KTSERSQPRRRQPI PKVRRPEGRTWADPGYPWPLYGNEGCGWAGWLLS P	100
	-----W-----A-----L-----	
	RGRSPSWGPTDPRRRSRNLOKVI DTLTCGFADLMGYI PLVGAPLGAARA	150
	LAHGVRVLEDGVNYATGNLPGCSFSI FLLALLSCLTVPASAYQVRNSTGL	200
	-----I-----E-----VS-I	
	YHVYNDPCFNSSI VYEANDAI LHTPGCVPCVREGNVSRCWAMTPTVATRD	250
	-----S-----A-M-M-----D-S-----L-----L-A-N	
	GKLPATQLRRHI DLLVGSATLCSALYVGDLCGSVFLI GQLFTFSPRRHW	300
	ASV-T-TI-----V-----A-AF-----M-----VS-----E-	
	TQGCNCISI YPGHI TOHRMAWDMMNWSP TAALVMAQLLRI PQAI LDMI AG	350
	V-D-----LS-----T-----VS-----VV-----V-	
	AHWGVLGI AYSFMYGNWAKVL VVLLLFAGVDAETI VSQGQAARMSGLV	400
	-----L-----A-----YT-----A-SHTT-T-A	
	SLFTFGAKQNI QLI NTNGSWHI NSTALNCNES LNTOWLAGLI YQHKFNSS	450
	-----S-----S-R-----V-----R-----D-H-----F-A-F-T-R-----	
(a)	GCPEKRLASCRRLTDFDQGWGPI SHANGSGPDORPYCWHYPPKPCGI VPAK	500
(b)	-----M-----IDW-A-----TYTEPDS-----A-R-----S	
(c)	-----Y-----	
	SVCOPVYCFTSP	550
	Q-----	
	-----VVVGTTDRS GAPTYSWGENDTDVFLNNTRPFLGNWF	600
	GCTWMNSTGFTKVCAPPFCVI GGAQNTLHCFTDCFRKHPDATYSRCGS G	650
	PWITPRCLVDYPIYLWHWPCTI NYTI FKI RMYVGGVEHRLAECNWTGE	700
	RCDEEDRDRS ELS P L L T T T T Q W Q L P C S F T T L P A L S T G L I H L H Q N I V D V Q	750
	YLYGVGSS I ASWAI KWEYVVL L F L L A D A R V C S C L W M M L L I S Q A E A L Q N	800
	LVI LNAASLAGTHGLVS FLVFFCFAYWLKGWVPGAVYTFYQMWPL L L L L	850
	LALPQRAYALDTEVAAS CGGVVLVGLMAL T L S P Y Y K R Y S W C L W W L Q Y F L	900
	TRVQAQLHVWIPFLNVRGGRDAVI L L M L A V H P T L V F D I T K L L L A V F G P L W	950
	I L Q A S L L K V P W F V R V Q G L L R F C A L A R K M I G Q H Y V Q M V I K L G A L T G T Y V Y	1000
	NH L T P L R D W A H N Q L R D L A V A V E P V V F S Q M E T K L I T W G A D T A A C O D I I N G L	1050
	P Y S A R R O R E I L L G P A D G M V S K G W R L L A P I T A Y A Q Q T R G L L O C I I T S L T G R	1100
	D K N O V E G E V Q I V S T A A Q T F L A T C I N O V C W T V Y H G A G T R T I A S P K G P V I Q M	1150
	Y T N V D Q D L V G W P A P Q G S R L T P C T C G S S D L Y L V T R H A D V I P V R R R G A S R G	1200
	S L L S P R O I S Y L K G S S O G P L L C P A G H A V G I F R A A V C T R G V A K A V D F I P V E N	1250
	L E T T M R S P V F T D N S S P P V P Q S F Q V A H L H A P T G S G K S T K V P A Y A A A Q Q Y K	1300
	V L V L N P S V A A T L G F G A Y M S K A H G I D P N I R T G V R T I T T G S P I T Y S T Y G K F L	1350
	A D G G C S G G A Y D I I C D E L H S T D A T S I L G I G T V L D Q A E T A G A R L V V L A T A T	1400
	P P G S V T V P H P N I E E V A L S T T G E I P F Y G K A I P L E V I K G G R H L I F C H S K K K C	1450
(a)	DELAALKLVALGI NAVAYYRGLDVS VI PTS GDVVVVATDALMTGYTGDFDS	1500
(c)	-----T-Y-----RR-----	
	VI DCNTCVTQTVDFSLDPTFTI ETI TLPQDAVS RTQRRORTGRGKPGI YR	

TABLE 6-continued

-----T-----A-----S-----L-----	1530
FVAPGERPSGMFDSVLCCEYDAGCAWYELTPAETT VRLRAYMNTFGLPV	1600
-----S-----A-----D-----F-----K-----	
CNDHLEFWEQVFTGLTHI DAHFLS QTKQS GENLPYL VAYQATVCARAQAP	1650
-----V-----I-----	
PPSWDOMWKCLIRLKP TLHGPTPLLYRLGAVQNEI TLTHPVT KYI MTCMS	1700
ADLEVVTSTWVL VGGVLAALAAAYCLSTGCVVI VGRVVLS GKPAI IPDREV	1750
LYREFDEMEECQHLPIYI ENGMMLAENFKQKALGLLQTAS RQAEVI APAV	1800
QTNWQKLETFWAKHMWNFI SGI QYLAGLSTLPONPAI ASLMAFTA AVTSP	1850
LTTSQTL LFNILGGWVAAQLAAPGAATAFVAGLAGAAI GS VOLGKVLID	1900
ILAGWGAGVAGALVAFKIMS GEVPTSTEDLVNLLPAI LSPGALVVGVC AA	1950
ILRRHVGPGE GAVNWMNRLI AFAS RGNHVS PTHYVPE SDAARVT AI LSS	2000
LTVTQLLRRLHQWISSECTTFC SOWLRDIWDWI CEVLSDFKTWLKAKLM	2050
PQLPGI PFVS CQRQYKGVWRVDGI MHTRCHCGAEI TGHVNGTMR I VGR	2100
TCRNWMSGTFPI NAYTTGPTPLPAPNYTFALWRVS AEEYVEI RQVGDHF	2150
YVTGMTTDLNLCPCQVPSPEFFTEL DGVRLHRFAPPCPKLLREEVSFRVG	2200
LHEYPVGSQLPCEPEPDAVLTSM L TDP SHI TAAAGRR LARGS PPSVAS	2250
SSASQLSAPSLKATCTANHDS PDAELI EANLLWRQEMGONI TRVESENKV	2300
VLDSFDPLVAEEDEREI SVP AEI LRKSRRFAQLPVWARP DYNPLVET	2350
WKKPDYEPV VHGCP LPPKSPVPPPRKRTVVLTESTLSTALAE LATR	2400
SFGSSSTSGITGDNNTTSS E PAPS GCPFDS DAESYS MPPL EOE P GDPDL	2450
SDGWSVTSS EANAEDVVCCSMS YSWT GALVTPCAAEEQKLPI NALS NSL	2500
LRHHNLVYSTTS RSACQKQKVT FDR LQV LDSHYQDVL KEVKAAAS KVKA	2550
NLSVEEACSLTPPHS AKS KFGYGAKDVRCHARKAVTHI NSVWKDLLEDN	2600
VTPIDTTI MAKNEVF CVQPEKGRKPARLI VFPDLGV RVCEKMALYDVVT	2650
KLP LAMVGSS YGFGYS PGQRVEFLVQAWKS KKT PMGFS YDTRCFDS TVTE	2700
SDI RTEAEI YQCCDLDPQARVAI KSLTERLYVGGPLTNS RGENGCGYRRCR	2750
ASRAS GVLTTSCGNTLT CYI KARAACRAAQLQDCTML VCGDDL VVI CES A	2800
GVQEDAA S LRAFTTEAMTRY S APFGDP PQPEYDELEI TS CS SNVS VAHDGA	2850
GKRVYLT RDPTTFLARA AWETARHTPVNSWLGNI IMFAPT LWARM LMY	2889
HFFSVLI ARDQLEQALDCEI YGACYSI EPLDLFPI I QRL	

Synthetic peptides have been increasingly used to map antigenic or immunogenic sites on the surface of proteins, an approach recently termed "site-directed-serology". The present inventor (Wang, C. Y.) and a colleague have taken this approach to identify and characterize highly antigenic epitopes on the envelope proteins of HIV and to develop sensitive and specific immunoassays for the detection of antibodies to HIV (previously designated HTLV-III) (19-21). See also U.S. Pat. No. 4,735,896, issued Apr. 3, 1988 and U.S. Pat. No. 4,879,212 issued Nov. 7, 1989, the contents of which are, hereby, fully incorporated by reference (22, 23). Subsequently, a series of finely mapped ell-characterized HTLV-1/II related synthetic peptides were employed in the development of synthetic peptide-based diagnostic assays for the detection of HTLV-1/II antibodies in infected individuals (24, 25). See also U.S. Pat. No. 4,833,071 issued May 23, 1989, U.S. Ser. No. 07/297,635 filed Jan. 13, 1989 and U.S. Ser. No. 07/469,294 filed Jan. 24, 1990. These assays have provided superior sensitivity, excellent specificity, and, in certain cases, an unmatched capability to differentiate infections with two closely related viruses, thus overcoming many of the existing problems associated with biologically-derived tests based on either viral lysate or recombinant DNA-derived protein.

It is, therefore, an objective of the present invention to develop a detection or diagnostic procedure to identify and monitor HCV infection early in the disease cycle.

Another objective is to develop a test procedure that is highly sensitive and accurate.

A further objective is to chemically synthesize a test reagent which can then be used to detect the presence of antibodies to HCV in body fluids and diagnose NANBH.

Another objective is to develop a vaccine which, when introduced into healthy mammals, including humans, will stimulate production of efficacious antibodies

to HCV, thereby providing protection against HCV infection.

A further objective is to provide a synthetic immunogen which can be used in mammals for the development of monoclonal and polyclonal antibodies to HCV.

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# BRIEF DESCRIPTION OF THE INVENTION

According to the present invention, a series of synthetic peptides representing immunodominant regions of the hepatitis C virus (HCV) proteins, each arranged in a specific sequence, has been identified and made by solid phase peptide synthesis. These peptides have been found to be useful in a highly sensitive and accurate method for the early detection of antibodies to HCV in sera and body fluids and the diagnosis of non-A non-B hepatitis (NANBH). Because of their high immunoreactivity, it is expected that these peptides are also useful in stimulating production of antibodies to HCV in healthy mammals such as Balb/C mice, and in a vaccine composition to prevent HCV or NANBH infection.

According to the present invention, a peptide composition useful for the detection of antibodies to HCV and diagnosis of NANBH comprises a peptide selected from the group of peptides with the following sequences:

- (i) Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X
- (ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Gln-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X
- (iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Gln-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X
- (iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Gln-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X
- (v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Gln-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X
- (vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Glu-Thr-Asn-Trp-Gln-Lys-Leu-Gln-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X
- (vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-

-continued  
Gln-Lys-Leu-Glu-Thr-X

(viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-  
Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-  
Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-  
Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-  
Arg-Gly-Asn-His-Val-Ser-Pro-X

(ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-  
Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-  
Glu-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-  
Glu-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-  
Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-  
Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-  
Ser-Glu-Pro-Arg-Gly-Arg-Arg-X, and

(x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-  
Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-  
Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-  
Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-  
Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

wherein X is —OH or —NH<sub>2</sub>, and analogues, segments, mixtures, combinations, conjugates and polymers thereof.

The amino acids in this application are abbreviated as shown herein below:

A = Ala = alanine,  
R = Arg = arginine,  
D = Asp = Aspartic acid,  
N = Asn = asparagine,  
Q = Gln = glutamine,  
E = Glu = glutamic acid,  
L = Leu = leucine,  
K = Lys = lysine,  
H = His = histidine,  
T = Thr = threonine,  
G = Gly = glycine,  
I = Ile = isoleucine,  
F = Phe = phenylalanine,  
S = Ser = serine,  
W = Trp = tryptophan,  
Y = Tyr = tyrosine,  
V = Val = valine,  
C = Cys = cysteine,  
P = Pro = proline

An example of a combination is: Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X wherein X is —OH or —NH<sub>2</sub>. An example of a segment of Peptide II is: Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X wherein X is —OH or —NH<sub>2</sub> (IIF). An example of a segment of Peptide III is: Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X wherein X is —OH or —NH<sub>2</sub> (IIID). An example of a segment of Peptide IX is: Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-

Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X (IXC).

The present invention also includes a highly sensitive and accurate method of detecting antibodies to HCV in body fluids and of diagnosing NANBH comprises the following steps:

A. Preparing a peptide composition comprising a peptide selected from the group having the following amino acid sequences:

(i) Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X

(ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Gln-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X

(iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X

(iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X

(v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X

(vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X

(vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X

(viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X

(ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Glu-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Glu-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X, and

(x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-

-continued  
 Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-  
 Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-  
 Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

wherein X is —OH or —NH<sub>2</sub>, and analogues, segments, mixtures, combinations, conjugates and polymers thereof; and

B. Using an effective amount of the peptide composition as the antigen in an immunoassay procedure.

Further, according to the present invention, the peptides by themselves, or when coupled to a protein or a polymeric carrier of homo or hetero dimers or higher oligomers by use of homo or hetero functional multivalent cross linking reagents, or when directly synthesized and conjugated to a branching polyvalent lysine resin, can be used to elicit the production of antibodies to HCV in healthy mammals, including humans.

The method comprises introducing an effective amount of the peptide composition containing each of the individual peptides, analogues or segments or a mixture or a combination thereof, or in a polymeric form, into the body of a healthy mammal by intraperitoneal or subcutaneous injection.

Vaccines containing the peptides according to the present invention as the key immunogen may also be prepared. It is expected that such vaccine compositions may be useful to prevent HCV infection or NANBH.

#### BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. 1-1, 1-2, 1-3 and 1-4 show the amino acid sequences of the immunodominant region of a HCV non structural protein and precisely delineates the amino acid residues (underlined to show - - - marginal, — moderate, and — strong) that contribute to the immunoreactivities of these HCV peptides with four representative HCV antibody positive sera. The immunoreactivities were measured as absorbance at 492 nm by an EIA procedure.

FIGS. 2-1 and 2-2 are comparisons of the signal to cutoff ratio between the peptide based HCV-EIA employing only the non-structural protein sequence derived Peptide IIG of the present invention and that of the recombinant SOD/HCV C-100-3 protein based HCV-EIA. In FIG. 2-1 a well-characterized HCV antibody positive control at various serum dilutions was used as the test sample. In FIG. 2-2 a panel of serum specimens derived from serial bleedings of a single individual spanning a period of sero-conversion to anti-HCV reactivity were used as samples.

FIGS. 3-1 and 3-2 depict the frequency distribution of the HCV-EIA positivity, using Peptide IIG, represented by the signal to cutoff ratios obtained with 264 normal serum and 264 normal plasma specimens from commercial sources. The mean s/c ratios for the negative (n=250) and screened out positive (i.e. n=14) serum specimens are 0.034 and 7.202 respectively; and for the negative (n=255) and positive (n=9) normal plasma specimens the mean s/c ratios are 0.084 and 7.089 respectively.

FIG. 4 is a histogram depicting the immunoreactivities of Peptide IIG with sera from individuals: (a) positive for HBsAg, (n=50); (b) positive for antibodies to HBc protein, (n=39); (c) with elevated (>100 I.U./L) alanine aminotransferase (ALT) enzyme activity, (n=174); (d) positive for antibodies to retroviruses HIV-1 (n=100), HIV-2 (n=10), HTLV-L/II (n=14); all asymptomatic, (total n=124); (e) with AIDS, ARC

(N=200) or ATL (n=170) disease, (total n=270); and (f) with autoimmune disease (n=20).

FIG. 5 provides a comparison between EIA results using the Peptides IIF and IID of the present invention and recombinant SOD/HCV C-100-3 as represented by their respective s/c ratios on a panel of repeatably reactive specimens (n=23) obtained from a random donor population.

FIG. 6 provides a comparison between a passive hemagglutination assay (PHA), using Peptide IIG, and the recombinant SOD/HCV C-100-3 EIA as represented by their respective P/C and s/c ratios for a panel of SOD/HCV C-100-3 HCV EIA repeatably reactive specimens (n=20) obtained from a random donor population. For results obtained by the PHA, the agglutination pattern is quantitated by a specially designed optical reading instrument (manufactured by Olympus Corporation) where a P/C ratio of larger than 20 is considered negative whereas a P/C ratio of less than 20 is considered positive.

FIG. 7-1 provides a study of serum samples collected over a ten year period of time from a NANBH patient who sero-converted after receiving HCV infected blood. The samples were tested by two EIA formats designated as A (coated with Peptides IIF and IID at 5 ug/mL each) and B (coated with Peptides IIF, IID and V at 5 ug/mL each) for comparison. The serum samples were provided by Dr. H. Alter of NIH.

FIG. 7-2 provides a kinetic study with serum samples, kindly provided by Dr. C. Stevens of New York Blood Center, from a hemodialysis patient who sero-converted and contracted NANBH. These were tested by EIA format B (coated with peptides IIF, IID and V at 5 ug/mL each).

FIG. 7-3 provides a second kinetic study with serum samples, kindly provided by Dr. D. Bradley of Center for Disease Control, from a chimpanzee which sero-converted after being inoculated with a well-characterized strain of HCV and contracted NANBH, also tested by EIA format B.

FIGS. 8-1 and 8-2 depict the signal/cutoff ratio frequency distribution of both negative and positive serum specimens by a HCV-EIA format B. The results were obtained using 2035 low risk random blood donor specimens screen tested in a blood bank setting.

FIG. 9 illustrates the inhibition by Peptide IV (an analogue) of binding of HCV specific antibodies to plates coated with peptides IID and IIF at 5 ug/mL each at various Peptide IV concentrations.

FIG. 10 provides a comparison between the peptide based HCV EIA (coated with Peptide IIF and V at 10 and 5 ug/mL respectively) and recombinant protein based HCV EIA using samples from 74 hemodialysis patients, kindly provided by investigators at the Japanese National Institute of Health.

FIGS. 11-1, and 11-2 show the amino acid sequences of an immunodominant region of the postulated HCV structural (core or nucleocapsid) protein and precisely delineates the amino acid residues that contribute to the immunoreactivities of these HCV peptides with four representative HCV antibody positive sera (Samples 1-4). The immunoreactivities were measured as absorbance at 492 nm by an EIA procedure.

FIG. 12-1, 12-2 and 12-3 are histograms depicting the frequency distribution of HCV positivity in 221 sera from individuals: (a) with AIDS, ARC (n=63); (b) positive for HBsAg, (n=50); (c) positive for antibodies to HBc protein, (n=22); (d) with elevated (>100

I.U./L) alanine aminotransferase (ALT) enzyme activity, (n=86) tested using three HCV EIA formats using Peptides IIH, V and VIIIIE at 5, 3, and 2 ug/mL respectively (Format C); Peptides VIIIIE, and DDX at 2 and 2 ug/mL each (Format D), and Peptides IIH and V at 5 and 3 ug/mL each (Format A).

FIGS. 13-1, 13-2, 13-3, 13-4, 13-5, and 13-6 depict the signal to cutoff ratio frequency distribution of HCV positivity in low risk random donor specimens using three HCV-EIA Formats, A (13-1 and 13 2), C (13-3 and 13-4), and D (13-5 and 13-6). The results were screen tested in a blood bank setting.

FIG. 14-1 provides a study of serum samples collected over a ten year period of time from a NANBH patient who sero-converted after receiving HCV infected blood. The samples were tested by a third EIA format designated as C (coated with Peptides IIH, V, and VIIIIE at 5, 3 and 2 ug/mL respectively) in comparison to two other EIA formats (designated as A and B.)

FIG. 14-2 provides another kinetic study with serum samples, kindly provided by Dr. D. Bradley of Center for Diseases Control, from a chimpanzee which sero-converted after being inoculated with a well-characterized strain of HCV and contracted NANBH. These samples were tested by the HCV EIA Format C, in comparison to a RIA using rDNA based HCV C-100 protein as the antigen. The ALT levels are also indicated with each bleed as a reference parameter.

FIGS. 15-1 and 15-2 both provide a side-by side data comparison via x-y plots with samples from hemodialysis patients, kindly provided by investigators at the Japanese National Institute of Health. The results were obtained by using the peptide based HCV EIA Format C (coated with peptides derived from both the structural and non-structural proteins containing IIH, V and VIIIIE at 5, 3, and 2 ug/mL respectively), HCV EIA Format A (coated with peptides derived from the non-structural protein region containing IIH and V at 5 and 3 ug/mL respectively), and the recombinant HCV C-100 protein based EIA.

The amino acids in the drawings and tables are abbreviated using the art accepted single letter codes as follows:

A=Ala=alanine,  
R=Arg=arginine,  
D=Asp=aspartic acid,  
N=Asn=asparagine,  
Q=Gln=glutamine,  
E=Glu=glutamic acid,  
L=Leu=leucine,  
K=Lys=lysine,  
H=His=histidine,  
T=Thr=threonine,  
G=Gly=glycine,  
I=Ile=isoleucine,  
F=Phe=phenylalanine,  
S=Ser=serine,  
W=Trp=tryptophan,  
Y=Tyr=tyrosine,  
V=Val=valine,  
C=Cys=cysteine,  
P=Pro=proline,  
M=Met=methionine

#### DETAILED DESCRIPTION OF THE INVENTION

In accordance with the present invention, three peptides and their segments have been chemically synthe-

sized for the detection of antibodies to HCV in body fluids, the diagnosis of NANBH, and for the vaccination of healthy mammals by stimulating the production of antibodies to HCV. These peptides are arranged in the following sequences:

- (i) Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X (I)
- (ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (II)
- (iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (III)
- (iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X (IV)
- (v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X (V)
- (vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X (VI)
- (vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X (VII)
- (viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X (VIII)
- (ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X, and (IX)
- (x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

wherein X is —OH or —NH<sub>2</sub>.



These peptides may comprise combinations or segments, i.e. longer or shorter peptide chains by having more amino acids added to the terminal amino acids, or by amino acids removed from either terminal end.

These peptides may also comprise analogues to accommodate strain-to-strain variations among different isolates of HCV. HCV is indicated to have frequent mutations. Therefore, it is expected that variant strains, such as J-1 and J-4 (see reference #27), exist. Adjustments for conservative substitutions and selection among the alternatives where non-conservative substitutions are involved, may be made in the prescribed sequences. It is expected that as long as the peptide's immunoreactivity recognizable by the antibodies to HCV is preserved, analogues of the synthetic peptide may also comprise substitutions, insertions and/or deletions of the recited amino acids of the above sequence.

These peptides may also comprise conjugates, i.e., they may be coupled to carrier proteins such as bovine serum albumin (BSA) or human serum albumin (HSA). Furthermore, these peptides may comprise polymers, i.e., they may be synthesized on a polymeric resin, such as a branching octameric lysine resin.

The amino acid sequences of the polypeptides useful as test reagents for the detection of antibodies to HCV in body fluids and diagnosis of NANBH are selected to correspond to a partial segment of the amino acid sequence of the HCV proteins: a non-structural protein designated as HCV C-100(18), and a structural protein such as the core (nucleocapsid) protein (27).

In selecting regions of the HCV protein for epitope analysis, peptides in the 40 mer size range with amino acid sequences covering the complete HCV C-100 protein and the postulated core protein were synthesized. These were tested for their immunoreactivity with serum from a patient positively diagnosed with HCV infection. Six overlapping peptides from the HCV C-100 protein region designated as I, II, III, IV, V and VI and two adjacent peptides form the postulated core protein region designated as VIII and IX were identified to have specific immunoreactivity with the positive HCV serum. Another peptide VII and its fragments, C-terminal to this immunodominant region, was also found to have moderate immunoreactivity with a sub population of HCV positive sera. See Example 12. Peptide IIH, another analogue of Peptide II, with five additional amino acids to the N-terminus has been found to be highly immunogenic and contains an additional epitope recognizable by antibodies in sera from patients with acute phase NANBHv infection (with elevated ALT levels). The amino acid sequences of the peptides are as follows:

- (i) Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X
- (ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Gln-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Gln-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X
- (iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Gln-Cys-Ser-Gln-His-Leu-

-continued

Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X

- (iii) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Gln-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X
- (iv) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Gln-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X
- (v) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X
- (vi) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X
- (vii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X
- (viii) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X, and
- (ix) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

The six peptides I, II, III, IV, V and VI span a region of 90 amino acids:

Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Gln-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe

- (ii) and were found to have specific immunoreactivity with the positive control serum. Table I shows the amino acid sequence of this immunodominant region of the HCV protein, and presents the amino acid sequence of the six chemically synthesized peptides, designated as I to VI and segments (A to H) thereof.

Another two peptides (VIII and IX) spanning a region of 119 amino acids located inside the 5' terminal of the postulated HCV core protein:

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-  
 Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-  
 Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-  
 Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-  
 Val-Arg-Alg-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-  
 Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-  
 Val-Arg-Arg-Pro-Gln-Gly-Arg-Thr-Trp-Alg-Gln-  
 Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-  
 Gly-Cys-Gly-Trp-Alg-Gly-Trp-Leu-Leu-Ser-Pro-  
 Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-  
 Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

were found to have specific immunoreactivity with a representative panel of well-characterized HCV antibody positive sera.

Table 7 shows the amino acid sequence of this immunodominant region of the postulated HCV core protein, and presents the amino acid sequence of the ten chemically synthesized peptides. They were designated, as Peptides VIII and IX with segments (A to D) thereof. Each of these peptides was coated at 5 ug/mL in a 10 mM sodium bicarbonate buffer (pH 9.5) onto polystyrene microwell plates and tested in a three step 45 minute enzyme immunoassay procedure, described hereinbelow, with a panel of HCV antibody positive sera, each selected as representative of a particular clinical population, at various serum dilutions.

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TABLE I  
CHARACTERIZATION OF THE IMMUNODOMINANT REGION OF THE HCV SUB-C100 FUSION POLYPEPTIDE:

CV, VIVGR	VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PYIEQ,GMMLA,EQFKQ,KALGL,LOTAS,ROAEV,IAPAV, QTNWQ,KLETF,WAKHM,WNF	RELATIVE (%) IMMUNOREACTIVITY
IA	GL,LOTAS,ROAEV,IAP	3.0
IB	KQ,KALGL,LOTAS,ROAEV,IAP	10.3
IC	MLA,EQFKQ,KALGL,LOTAS,ROAEV,IAP	23.9
ID	EQ,GMMLA,EQFKQ,KALGL,LOTAS,ROAEV,IAP	24.6
IE	HL,PTIEQ,GMMLA,EQFKQ,KALGL,LOTAS,ROAEV,IAP	38.2
IF	EE,CSQHL,PTIEQ,GMMLA,EQFKQ,KALGL,LOTAS,ROAEV,IAP	45.6
IIA	GMMLA,EQFKQ,KALGL	3.1
IIB	PTIEQ,GMMLA,EQFKQ,KALGL	24.3
IIC	CSQHL,PTIEQ,GMMLA,EQFKQ,KALGL	41.7
IID	DEMEE,CSQHL,PTIEQ,GMMLA,EQFKQ,KALGL	44.9
IIE	LYREF,DEMEE,CSQHL,PTIEQ,GMMLA,EQFKQ,KALGL	57
IIF	PDREV,LYREF,DEMEE,CSQHL,PTIEQ,GMMLA,EQFKQ,KALGL	99
IIG	IL,PDREV,LYREF,DEMEE,CSQHL,PTIEQ,GMMLA,EQFKQ,KALGL	93.2
IIH	SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PTIEQ,GMMLA,EQFKQ,KALGL	101
IIIA	EF,DEMEE,CSQHL,PTI	4.9
IIIB	EV,LYREF,DEMEE,CSQHL,PTI	26.3
IIIC	IL,PDREV,LYREF,DEMEE,CSQHL,PTI	85
IIID	SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PTI	100
IIIE	VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PTI	99
IIIF	VVLSG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PTI	95
IIIA	SG,KPAII,PDREV,LYREF,DEMEE,CSQHL,PTIEQ,GMMLA,EQF	93.8
VIA	AS,ROAEV,IAPAV, QTNWQ,KLET	3.9
VIB	GL,LOTAS,ROAEV,IAPAV, QTNWQ,KLET	43.6
VIC	KQ,KALGL,LOTAS,ROAEV,IAPAV, QTNWQ,KLET	44.0
VID	LA,EQFKQ,KALGL,LOTAS,ROAEV,IAPAV, QTNWQ,KLET	46.0
VIE	EQ,GMMLA,EQFKQ,KALGL,LOTAS,ROAEV,IAPAV, QTNWQ,KLET	34.8
VA	AV, QTNWQ,KLETF,WAKHM,WNF	1.3
VB	EV,IAPAV, QTNWQ,KLETF,WAKHM,WNF	17.8
VC	AS,ROAEV,IAPAV, QTNWQ,KLETF,WAKHM,WNF	23.4
VD	GL,LOTAS,ROAEV,IAPAV, QTNWQ,KLETF,WAKHM,WNF	32.3
V	KQ,KALGL,LOTAS,ROAEV,IAPAV, QTNWQ,KLETF,WAKHM,WNF	93.9

The underlined amino acid residues exert ( — ) marginal, ( — ) moderate, or ( — ) strong immunoreactivity

**TABLE 7**  
**Identification and Characterization of An Immunodominant Region in HCV's Structural Proteins, based on the predicted amino acid sequence derived from the combination of synthetic peptides (VIII-A-E and IX-A-E):**

[illegible]

Calculations based on the overall EIA absorbance of all positive sera yielded an array of immunoreactivity indices represented as % relative immunoreactivity for each of the synthetic HCV peptides. Three peptides, designated as IIF, IIH and IIID, being 40 mer, 47 mer and 30 mer in size, with the following amino acid sequence respectively:

(IIF)  
Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-  
Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-  
Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu

(IIH)  
Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-  
Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-  
Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Glu-Gly-  
Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-  
Leu-Gly-Leu

and

(IIID)  
Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-  
Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-  
Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile

were found to have the highest immunoreactivity with the sera panel. The relative (%) immunoreactivity for each of the 40 HCV peptides listed in Tables 1 and 7, as a result of this extensive epitope mapping study, provides a basis for the delineation of several clusters of amino acid residues (as underlined), each in a prescribed sequence, that are involved in or relevant to the antigenic configuration of the HCV peptides. Two peptides, designated as VIIIIE and IXD being 61 mer and 56 mer in size are respectively located within the HCV structural core protein region with the following amino acid sequences:

(VIIIIE)  
Ser-Thr-His-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-  
Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-  
Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-  
Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-  
Val-Arg-Alg-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-  
Gln-Pro-Arg-Gly-Arg-Arg-X

(IXD)  
Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Tyr-  
Trp-Alg-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-  
Gly-Asn-Glu-Gly-Cys-Gly-Trp-Alg-Gly-Trp-Leu-  
Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-  
Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-  
Gly-X

Peptides XIIIIE and IXD were also found to have the highest reactivity in this region.

Assays for antibodies to HCV based upon chemically synthesized peptides show several advantages over assays utilizing biologic based immunoadsorbents. The peptides can easily be synthesized in gram quantities by using automated solid-phase methods, thus providing a reproducible antigen of high integrity with consistent yields. The presence of other antigens from biological systems precludes such reproducibility. More importantly, non-specific reactivities seen in uninfected individuals are likely to be due to the heterogeneity of the preparations used for assay. This is particularly true for assays using biologically based immunoadsorbents. In these processes, the host antigens are frequently copurified with the desired viral protein(s). Antibodies to these contaminating antigens are frequently found in

normal individuals, thus resulting in false-positive results.

The assay of the present invention clearly minimizes such false-positive reactions as encountered in the other assay systems and, at the same time, shows a high sensitivity to truly positive sera by the substantially increased signal-to-noise ratio. This increased signal-to-noise ratio probably resulted from the purity of the immunoadsorbent. The assay of the present invention is also highly specific, in that the mean S/C ratios for HCV carriers are about 80-200 times the mean S/C of those of the non-infected individuals. For a representative example, see FIGS. 3-1 and 3-2.

The peptides useful as solid phase immunoadsorbents for the detection of antibodies to HCV were synthesized by the "classical" Merrifield method of solid phase peptide synthesis using slide chain protected t-Boc-amino acids to correspond to the following amino acid sequences:

- (i) Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X
- (ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X
- (iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X
- (iv) Cys-Val-Val-Ile-Val-Oly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X
- (v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X
- (vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X
- (vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X
- (viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X
- (ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-

-continued

Thr-Lys-Arg-Asp-Thr-Asn-Arg-Arg-Pro-  
 Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-  
 Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-  
 Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-  
 Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-  
 Ser-Gln-Pro-Arg-Gly-Arg-Arg-X, and

(IX)

(x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-  
 Arg-Arg-Pro-Gly-Gly-Arg-Thr-Trp-Ala-  
 Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-  
 Gly-Asn-Gly-Gly-Cys-Gly-Trp-Ala-Gly-  
 Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-  
 Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-  
 Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

wherein X is —NH<sub>2</sub>.

Other analogues, segments and combinations of these peptides may be prepared by varying the amino acid sequences either by adding, subtracting, substituting, or deleting desired t-Boc-amino acid(s).

Following completion of assembly of the desired blocked peptide on the resin, the peptide-resin is treated with anhydrous hydrofluoric acid to cleave the peptide from the resin. Functional groups of amino acids which are blocked during synthesis by benzyl-derived blocking groups are also cleaved from the peptide simultaneously. The free peptide is then analyzed and purified by high performance liquid chromatography (HPLC) and characterized biochemically by amino acid analysis.

Longer peptides with more than about 50 amino acids may also be prepared conveniently using well known recombinant methods. The known nucleic acids codons for each of the amino acids in the peptide may be utilized and synthetic genes encoding such peptides constructed. The synthetic gene may be inserted into vector constructs by known techniques, cloned and transfected into host cells, such as *E. coli*, or yeast. The secreted polypeptide may then be processed and purified according to known procedures. The peptides synthesized according to the above described procedures are highly reactive with antibodies to HCV and can be used as a highly sensitive and specific immunoadsorbent for the detection of antibodies to HCV.

FIGS. 1-1, 1-2, 1-3 and 1-4 and FIGS. 11-1 and 11-2 show the amino acid sequences of the immunodominant regions of HCV proteins, both structural and non-structural, and precisely delineates, in the case of the non-structural protein HCV C-100 region, the underlined amino acid residues that contribute (--- marginally, — moderately, or — strongly) to the immunoreactivities, measured at A492 nm by a peptide based EIA procedure of these HCV peptides with four representative HCV antibody positive sera.

The peptide based EIA procedure used to measure the immunoreactivity of each peptide is as follows. 100 uL per well of each of the peptides was coated at 5 ug/mL in a pH 9.5 sodium bicarbonate buffer (10 mM) onto a polystyrene microwell plate and the microwell plate was incubated at 37° C. for about an hour, washed and dried. The test serum samples were diluted with PBS containing normal goat serum, gelatin and TWEEN 20. 200 uL of the test serum sample solution was added to each well and allowed to react for 15 mins. at 37° C. The wells were washed, enzyme labelled antibodies were used to bind the HCV-antibody-peptide complex, and the plate was incubated for another 15 min. A color developer, e.g. orthophenylenediamine (OPD), was then added. The reaction was stopped after 15 min by the addition of 50 uL 1.0M H<sub>2</sub>SO<sub>4</sub>, and the

absorbance of the reaction mixture was read at 492 nm with an ELISA reader.

As demonstrated in FIG. 1-1, serum sample 1 has little reactivity with Peptide 1A and 1B. However, its reactivity with Peptide 1C increases significantly, followed by a marginal increase with Peptide 1D, and additional increases with Peptides 1E and 1F. This indicates that in the HCV Peptide I series, two clusters of amino acid residues, namely Leu-Ala-Glu-Gln-Phe and LAEQF and His-Leu-Pro-Tyr-Ile and HLPYI and, are contributing to the antigenic determinant(s) of the HCV Peptide I. Similarly, a cluster of residues namely Glu-Glu-Cys-Ser-Glu-His-Leu-Pro-Tyr-Ile and EECSQH-LPYI and is contributing to the immunoreactivity of the HCV Peptide II series; another cluster of residues namely Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg and SGKPAIIPDR and is contributing to the immunoreactivity of HCV Peptide III series and two clusters of residues, namely Gly-Leu-Leu-Glu-Thr and GLLQT and and Glu-Val-Ile-Ala-Pro and EVIAP and are contributing to the immunoreactivity by HCV Peptides IV and V series. As shown on the bottom of FIG. 1-1, a total of six spaced clusters of amino acid residues representing discontinuous epitopes in this immunodominant region of the HCV protein are identified as contributing to the specific HCV immunoreactivity with serum sample 1.

FIG. 1-2 illustrates an immunoreactivity profile for serum sample 2 when tested on a total of 31 overlapping peptides in the HCV Peptide I, II, III, IV, V and VI series. There is a clear difference between the immunoreactivity profiles of serum samples 1 and 2. The immunodominant epitope, as marked by residues Ser-Gly-Lys-Pro-Ala and SGKPA and Ile-Ile-Pro-Pro-Asp-Arg-Glu-Val and IIPDREV and, is located towards the N-terminus of the region.

FIG. 1-3 illustrates an immunoreactivity profile for serum sample 3 when tested on the same 31 HCV peptide panel. Through this extensive epitope mapping analysis, serum sample 3 was found to have a similar immunoreactivity profile to that of serum sample 2.

FIG. 1-4 illustrates an immunoreactivity profile for serum sample 4 which differs significantly from that of sample 2 and 3, while maintaining some similarity to that of sample 1.

In summary, epitope mapping analysis conducted with a series of 31 overlapping peptides covering an immunodominant region of the HCV non-structural protein, which spans a total of 90 amino acid residues as illustrated in Table 1, and an immunodominant region of the HCV structural core protein, which spans a total of 119 amino acid residues as illustrated in Table 7, reveals a varying degree of immunoreactivity among different HCV antibody positive samples and these HCV peptides. Based on overall EIA absorbance readings obtained with a panel of eight HCV positive sera with each of these 31 HCV peptides (Table 2), a relative (%) immunoreactivity index is established for each of the peptides and several clusters of amino acid residues are identified as contributing strongly, as in the cases of Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg and Glu-Val-Ile-Ala-Pro; moderately, as in the cases of Ser-Gly-Lys-Pro-Ala, Glu-Val-Leu-Tyr-Arg-Glu-Phe, Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly; and Leu-Ala-Glu-Gln-Phe-Lys-Gln; or marginally, as in the case of Lys-Gln-Lys-Ala-Leu, to the HCV immunoreactivity.

Similarly, the relative immunoreactivity of Peptide VIII and IX and their analogue-segments are presented in Table 7.

normal plasma specimens from commercial sources. The mean s/c ratios for the negative (n=250) and screened out positive (i.e. n=14) serum specimens are

TABLE 2

HCV Peptide Segments													
Specimens													
I							II						
A	B	C	D	E	F	G	A	B	C	D	E	F	G
Blank	0.041	0.041	0.041	0.041	0.041	0.041	0.041	0.040	0.040	0.041	0.041	0.041	0.044
NRC	0.047	0.050	0.049	0.049	0.052	0.053	0.045	0.044	0.048	0.046	0.048	0.088	0.074
WRC	0.040	0.048	0.077	0.084	0.155	0.221	0.040	0.042	0.220	0.153	0.341	0.399	0.363
SRC	0.049	0.055	0.330	0.383	0.828	1.175	0.043	0.093	1.188	0.963	1.279	1.832	1.672
1	0.066	0.218	1.925	2.151	2.994	3.247	0.075	0.838	3.219	3.282	3.494	3.316	3.395
2	0.054	0.095	0.080	0.093	0.171	0.337	0.066	0.103	0.243	0.536	0.872	2.929	2.746
3	0.062	0.089	0.062	0.064	0.068	0.108	0.065	0.058	0.121	0.129	0.371	2.406	2.696
4	0.082	1.068	1.391	1.912	1.994	2.726	0.074	2.769	2.387	2.437	2.822	3.289	3.169
5	0.063	0.083	0.136	0.156	0.246	0.216	0.057	0.065	0.104	0.085	0.197	0.732	0.261
6	0.059	0.073	0.058	0.066	0.071	0.071	0.061	0.068	0.066	0.061	0.086	0.623	0.488
7	0.050	0.052	0.058	0.062	0.091	0.091	0.046	0.049	0.066	0.048	0.152	1.146	1.100
8	0.070	0.087	0.254	0.293	0.710	0.698	0.070	0.076	0.718	0.812	1.463	1.998	1.624
8	0.056	1.765	3.964	4.077	6.345	7.494	0.514	4.026	6.924	7.390	9.457	16.44	15.48
i = 1	A 492 nm												
% Relative Immunoreactivity	3.0	10.3	23.9	24.6	38.2	45.6	3.1	24.3	41.7	44.9	57	99	93.2

Specimens						
III						
A	B	C	D	E	F	
Blank	0.040	0.046	0.040	0.045	0.040	0.043
NRC	0.44	0.048	0.061	0.073	0.071	0.070
WRC	0.046	0.043	0.192	0.243	0.269	0.222
SRC	0.046	0.074	1.081	1.260	1.379	1.127
1	0.289	0.527	3.245	4.057	3.545	3.613
2	0.191	0.316	2.715	2.941	3.053	2.984
3	0.066	0.085	2.407	2.612	2.566	2.624
4	0.064	2.864	3.096	3.221	3.319	3.220
5	0.056	0.126	0.588	0.657	0.700	0.522
6	0.054	0.075	0.458	0.623	0.641	0.489
7	0.045	0.273	0.863	1.577	1.669	1.505
8	0.058	0.101	0.655	0.894	0.937	0.820
8	A 492 nm	0.823	4.367	14.03	16.58	15.78
i = 1						
% Relative Immunoreactivity	4.9	26.3	85	100	99	95

Based on the above-mentioned epitope mapping study, four representative EIAs were configured using Peptide IIG alone, a mixture of two Peptides IIF and IIID, a mixture of IIF, IIID and V, or a mixture of IIF and V as the solid phase antigen.

FIGS. 2-1 and 2-2 depict the comparison, by signal to cutoff ratio, between the peptide based HCV-EIA employing Peptide IIG, at 5 ug/mL coating concentration, and that of recombinant SOD/HCV C-100-3 protein based HCV-EIA. In FIG. 2-1, a well-characterized HCV antibody positive control at various serum dilutions was used as the sample. In FIG. 2-2, a panel of serum specimens derived from serial bleedings of a single individual spanning a period of sero-conversion to anti-HCV reactivity was used. Similar dilution titers and equal ability to identify date of sero-conversion, the two parameters indicative of the sensitivity of each assay, are obtained with the synthetic peptide based EIA according to the present invention and rDNA HCV C-100 based EIA, except that the peptide based assay according to the present invention is more sensitive, conferring a higher signal to cutoff ratio to its positive specimens.

FIG. 3-1 and 3-2 depict the frequency distribution of the synthetic peptide based HCV-EIA signal to cutoff ratios, using Peptide IIG at 5 ug/mL as the coating concentration, obtained with 264 normal serum and 264

0.034 and 7.202 respectively; for the negative (n=255) and positive (n=9) normal plasma specimens the mean ratios are 0.084 and 7.089 respectively. A sharp contrast between the screened out positives and all the negatives is obtained with the peptide based HCV-EIA of the present invention.

Based on the high degree of sensitivity and specificity of the peptide compositions according to the present invention in their immunoreactivities to antibodies to HCV, it is believed that the peptide compositions according to the present invention may also be useful as vaccines to prevent NANBH, and as immunogens for the development of both monoclonal and polyclonal antibodies to HCV in mammals, including humans. The peptide compositions when coupled to a protein, or synthesized on a polymeric carrier resin (e.g., an octameric branching lysine resin) or when polymerized to homo or hetero dimers or higher oligomers by cysteine oxidation, induced disulfide cross linking, or by use of homo or hetero functional multivalent cross linking reagents, can be introduced to normal subjects to stimulate production of antibodies to HCV in healthy mammals.

The advantages of using the peptides according to the present invention are many.

Since the peptide compositions according to the present invention are not derived biologically from the virus, there is no danger of exposing the normal subjects who are to be vaccinated to the disease.

The peptides can be chemically synthesized easily. This means that there is no involvement with the HCV at any time during the process of making the test reagent or the vaccine. Another problem which can be minimized by the process of the present invention is the false positive results caused by the presence of antigenic materials from host cells co-purified with the HCV fusion protein. Certain normal individuals have antibodies to *E. coli* or yeast proteins which are cross reactive with the antigenic materials from host cells. Sera from these normal individuals may show a positive response in the immunoassays.

Further, with appropriate amino acid modifications or substitutions, it is expected that various peptide analogues based on the prescribed amino acid sequence can be synthesized with properties giving rise to lower background readings or better binding capacity to solid phases useful for HCV antibody screening assays.

Moreover, because the peptide compositions of the present invention are synthetically prepared, the quality can be controlled and as a result, reproducibility of the test results can be assured. Also, since very small amounts of peptides are required for each test procedure, and because the expense of preparing the peptides is relatively low, the cost of screening body fluids for antibodies to HCV, diagnosis of NANBH infection, or the preparation of a vaccine is relatively low.

The peptides prepared in accordance with the present invention can be used to detect HCV infection and diagnose NANBH by using them as the test reagent in an enzyme-linked immunosorbent assay (ELISA), an enzyme immunodot assay, an agglutination based assay, or other well-known immunoassay devices. The preferred method is ELISA. The ELISA technique is exemplified in Examples 1, 2, 8-10, 12 and 14-18 and the agglutination based assay in Examples 3 and 4. The Examples are used to illustrate the present invention and are not to be used to limit the scope of the invention.

It is to be noted that in the following methods, 0.25% by weight of glutaraldehyde may be added to the coating buffer to facilitate better peptide binding onto the plates or beads. Further, horseradish peroxidase (HRPO) conjugated mouse monoclonal anti-human IgG antibody or the HRPO conjugated second antibodies from any other animal species may be used in place of the HRPO-conjugated goat anti-human IgG as the second antibody tracer.

The gelatin used in these processes can include calf skin gelatin, pig skin gelatin, fish gelatin or any known available gelatin proteins, or be replaced with albumin proteins.

#### EXAMPLE 1

Measurement of Relative (%) Immunoreactivity for synthetic peptide covering an immunodominant region of the HCV protein C-100 by an Enzyme-Linked Immunosorbent Assay

Wells or 96-well plates were coated at 4° C. overnight (or 1 hour at 37° C.), with each of the thirty one peptides: IA to IF, IIA to IIF, IIIA to IIF, IV, V, VIA to VIE (see Table 1) prepared as described at 5 ug/mL at 100 uL per well in 10 mM NaHCO<sub>3</sub> buffer, pH 9.5. The peptide coated wells were then incubated with 250

uL of 3% by weight of gelatin in PBS at 37° C. for 1 hour to block non-specific protein binding sites, followed by three washes with PBS containing 0.05% by volume of TWEEN 20 and then dried. The test specimens were diluted with PBS containing 20% by volume normal goat serum, 1% by weight gelatin and 0.05% by volume TWEEN 20 at dilutions of 1:20 volume to volume, respectively. 200 uL of the diluted specimens were added to each of the wells and allowed to react for 15 minutes at 37°.

The wells were then washed six times with 0.05% by volume TWEEN 20 in PBS in order to remove unbound antibodies. Horseradish peroxidase conjugated goat anti-human IgG was used as a second antibody tracer to bind with the HCV antibody-peptide antigen complex formed in positive wells. 100 uL of peroxidase labeled goat anti-human IgG at a dilution of 1:1800 in 1% by volume normal goat serum, 0.05% by volume TWEEN 20 in PBS was added to each well and incubated at 37° C. for another 15 minutes.

The wells were washed six times with 0.05% by volume TWEEN 20 in PBS to remove unbound antibody and reacted with 100 uL of the substrate mixture containing 0.04% by weight orthophenylenediamine (OPD) and 0.12% by volume hydrogen peroxide in sodium citrate buffer, pH 5.0.

This substrate mixture was used to detect the peroxidase label by forming a colored product. Reactions were stopped by the addition of 100 uL of 1.0M H<sub>2</sub>SO<sub>4</sub> and the absorbance measured using an ELISA reader at 492 nm (i.e. A<sub>492</sub>). Assays were performed in singlet at one specimen dilution (1:20) with a panel of eight representative HCV antibody positive sera, along with the specimen diluent blank, non-reactive, weakly reactive and strongly reactive controls (NRC, WRC, SRC) all in duplicates.

Results obtained from this study are shown in Table 2. According to the EIA absorbance readings at 492 nm (y axis) and the amino acid sequences for each of the corresponding HCV peptides (x axis), representative immunoreactivity profiles are plotted for four of the eight sera as shown in FIGS. 1-1 to 1-4. Relative (%) immunoreactivity index for each of the 31 peptides is calculated using Peptide IIID as a reference based on the total absorbance of eight sera at 492 nm (See Tables 1 and 2). FIG. 1 shows the amino acid sequences of the immunodominant region according to data presented in Tables 1 and 2, and precisely delineates the amino acid residues (underlined) that contribute (--- marginally, — moderately, and — strongly) to the immunoreactivities.

In summary, epitope mapping analysis conducted with a series of 31 overlapping peptides covering an immunodominant region of HCV, spanning a total of 90 amino acid residues as illustrated in Table 1, reveals a varying degree of immunoreactivities between different HCV antibody positive samples and these HCV peptides. Based on this study, several discontinuous epitopes are located within this immunodominant region. Contrary to what is speculated by the conventional wisdom, it is found preferably to have peptides with longer amino acid chains, ideally longer than 20, synthesized in order to optimally present these antigenic determinants to HCV antibodies.

Based on the above-mentioned epitope mapping study, four representative EIAs using peptide IIG alone, or a mixture of Peptides IIF and IID, or a mixture of IIF, IIID and V, or a mixture of IIF and V as the



solid phase antigen were configured for the following efficacy studies as demonstrated in Examples 2, 8, 9, 10 and 12.

### EXAMPLE 2

#### Detection of Antibodies to HCV by an Enzyme-Linked Immunosorbent Assay

Wells of 96-well plates were coated at 4° C. overnight (or for 1 hour at 37° C.) with either Peptide IIG alone at a coating concentration of 0.5 ug per well (designated as IIG EIA) or with a mixture of two Peptides IIF and IIID (designated as IIF/IIID EIA) in a ratio by weight of IIF:IIID=1:1 at 1 ug per well of the mixture in 100 uL 10 mM NaHCO<sub>3</sub> buffer pH 9.5. The peptide coated wells were then incubated with 250 uL of 3% by weight of gelatin in PBS at 37° C. for 1 hour to block non-specific protein binding sites, followed by three more washes with PBS containing 0.05% by volume of TWEEN 20 and dried.

The test specimens were diluted with PBS containing 20% by volume normal goat serum, 1% by weight gelatin and 0.05% by volume TWEEN 20 at dilutions of 1:20 volume to volume, respectively. 200 uL of the diluted specimens were added to each of the wells and allowed to react for 15 minutes at 37°.

The wells were then washed six times with 0.05% by volume TWEEN 20 in PBS in order to remove unbound antibodies. Horseradish peroxidase conjugated goat anti-human IgG was used as a second antibody tracer to bind with the HCV antibody-peptide antigen complex formed in positive wells. 100 uL of peroxidase labeled goat anti-human IgG at a dilution of 1:1800 in 1% by volume normal goat serum, 0.05% by volume TWEEN 20 in PBS was added to each well and incubated at 37° C. for another 15 minutes.

The wells were washed six times with 0.05% by volume TWEEN 20 in PBS to remove unbound antibody and reacted with 100 uL of the substrate mixture containing 0.04% by weight orthophenylenediamine (OPD) and 0.12% by volume hydrogen peroxide in sodium citrate buffer, pH 5.0. This substrate mixture was used to detect the peroxidase label by forming a colored product. Reactions were stopped by the addition of 100 uL of 1.0M H<sub>2</sub>SO<sub>4</sub> and the absorbance measured using an ELISA reader at 492 nm (i.e. A<sub>492</sub>). Assays were performed in singlet at one specimen dilution (1:20) with all test specimens. Each plate run is accompanied by a panel of eight controls including the specimen diluent blank, negative, weak HCV reactive and strong HCV reactive controls, all in duplicate. The strongly reactive control was adjusted by diluting a HCV positive serum in the specimen dilution buffer at 1:300, which gave an absorbance value at 492 nm of about 1.5 when performed in this standard 45 minute assay procedure. A cutoff value is calculated based on

the following formula: Cutoff=(0.1×SRC)+NRC. Both the raw absorbance (designated as signal) and the ratio of signal to cutoff are recorded for all specimens analyzed.

The following groups of specimens were analyzed on the HCV peptide based EIA according to the present invention, with the plates coated either with 5 ug/mL of peptide IIG or a mixture containing 5 ug/mL IIF and 5 ug/mL IIID:

- (a) A well-characterized HCV antibody positive control based on serum dilutions; (on both IIG and IIF/IIID EIAs)
- (b) a panel of serum specimens derived from serial bleedings of a single individual spanning a period of zero-conversion to anti-HCV reactivity; (on both IIG and IIF/IIID plates)
- (c) 264 normal serum and 264 normal plasma specimens from commercial sources; (on IIG plates only)
- (d) individuals positive for HBsAg, (n=30); (on both IIG and IIF/IIID plates)
- (e) individuals positive for antibodies to HBe protein, (n=39); (on both IIG and IIF/IIID plates)
- (f) individuals with elevated (>100 I.U./L.) alanine aminotransferase (ALT) enzyme activity, (n=174); (on both IIG and IIF/IIID plates)
- (g) individuals positive for antibodies to retroviruses HIV-1(n=100), HIV-2(n=10), HTLV-1/II(n=14); all asymptomatic, (total n=124); (on both IIG and IIF/IIID plates)
- (h) individuals with AIDS, ARC(n=200) or ATL (n=170) disease, (total n=370); (on both IIG and IIF/IIID plates) and
- (i) individuals with autoimmune disease (n=20). (on IIG plates only)
- (j) recombinant SOD/HCV C-100-3 HCV-EIA repeatedly reactive specimens obtained from a random donor population, (n=23). (on both IIG and IIF/IIID plates).

Results obtained from groups (a) and (b) are presented in FIGS. 2-1 and 2-2 respectively (data obtained on IIG plates only), from group (c) in FIGS. 3-1 and 3-2; from groups (d) to (i) in FIG. 4, from group (j) in Table 3 and FIGS. 5 and 6.

In brief, as shown in FIGS. 2-1 and 2-2, a comparison, by signal to cutoff ratio, between the peptide based HCV-EIA of the present invention employing peptide IIG and that of recombinant SOD/HCV C-100-3 protein based HCV-EIA produced by Chiron/Ortho. Similar dilution titers and equal ability to identify date of sero-conversion, the two parameters indicative of each assay's sensitivity, are obtained for both assays. However, the assay according to the present invention is more sensitive and confers a higher signal to cutoff ratio to its positive specimens.

TABLE 3

SAMPLE ID No.		rDNA				Anti-HBc (S/C)	OTHER POSITIVES	Peptide HCV-EIA S/C
		HCV S/C	RPT S/C	RPT S/C	ALT (IU/L)			
1	161	5.33	5.56	5.56	36/56	2.10		11
2	280	5.76	5.56	5.56	78/56	0.07+	HBc, ALT	10
3	374	1.98	2.45	2.45	20/56	1.97		0.573
4	517	5.79	5.68	5.68	34/56	2.04		11
5	561	1.74	2.75	2.47	21/56	2.46		0.172
6	675	0.93	1.33	1.54	29/56	1.98		0.135
7	720	5.68	5.68	5.68	57/56	0.08+	HBc, ALT	13
8	773	5.56	5.88	5.88	86/56	2.07	HIV, ALT	8.625
9	797	3.79	4.35	4.29	74/56	0.38+	HBc, ALT	1.802
10	869	5.66	5.59	5.59	35/56	2.45		9.755

TABLE 3-continued

SAMPLE ID No.	rDNA				Anti-HBc (S/C)	OTHER POSITIVES	Peptide HCV-EIA S/C
	HCV S/C	RPT S/C	RPT S/C	ALT (IU/L)			
11 873	5.66	5.59	5.59	26/56	2.34		1.189
12 1003	1.63	1.24	1.01	31/56	2.02		0.078
13 1073	5.73	5.59	5.59	17/56	0.12+	HBc	2.594
14 1099	1.72	1.76	1.94	10/56	1.84		0.083
15 1118	5.59	5.79	5.79	10/56	0.31+	HBc	10.5
16 1336	0.93	1.38	1.38	18/56	2.15		0.010
17 1501	5.75	5.67	5.67	36/56	1.99		5.349
18 1530	1.27	1.48	1.50	23/56	2.30		0.943
19 1557	0.91	1.29	1.28	20/56	2.20		0.385
20 1652	2.06	2.64	2.72	42/56	1.72		0.135
21 1877	5.59	5.63	5.63	65/56	2.16	ALT	4.943
22 1940	1.64	1.47	1.17	29/56	2.35		0.052
23 2017	5.60	5.84	5.84	11/56	0.19+	HBc	6.786

As shown in FIGS. 3-1 and 3-2, the frequency distribution of the HCV-EIA signal to cutoff ratios, using peptide IIG at 5 ug/mL as the coating concentration, that was obtained with 264 normal serum and 264 normal plasma specimens for commercial sources suggested a repeatably reactive rate of 5.3% and 3.4% respectively. These percentages are relatively high compared with those reported in field clinical trials (usually 0.5-1.0%) using the rDNA HCV C-100 based EIA kit (Chiron/Ortho). However, in the assay according to the present invention, the mean s/c ratios for the negative (n=250) and screened out positive (i.e. n=14) serum specimens are 0.034 and 7.202 respectively; for the negative (n=255) and positive (n=9) normal plasma specimens the mean ratios are 0.084 and 7.089 respectively. Such a sharp contrast between the screened out positives and all the negatives probably precludes the likelihood of a high false positive rate. Since these normal specimens are derived from commercial plasma centers where the paid donors usually represent a population with higher incidence of viral markers than the rigorously monitored blood banks, a higher repeatably reactive rate is also considered reasonable. Previous clinical studies indicated that between 7 to 10 percent of patients receiving transfusions developed NANBH, where 90% of these post-transfusion hepatitis cases are caused by the NANBHV(5). These reports also provide some support to the interpretation of the data obtained herein that a high reactivity represents a true positive result.

Results obtained from the screening of a total of 677 well-characterized clinical specimens previously categorized into six groups, from (d) to (i) using a representative lot of plates coated with Peptide IIG, were plotted on a histogram as shown in FIG. 4.

Fifteen out of fifty (i.e. 30%) HBsAg carriers, 3 out of 39 (i.e. 8%) HBc antibody positive individuals, 43 out of 174 (i.e. 24.7%) individuals with elevated ALT enzyme activity, 8 out of 124 (6.5%) asymptomatic individuals with retroviral antibodies, 6 out of 270 (i.e. 2.2%) individuals with retroviral related disease, and 0 out of 20 (i.e. 0%) individuals with autoimmune disease were found to be repeatably reactive with the peptide HCV EIA of the present invention using peptide IIG. All these positive specimens were also found to be positive when tested on peptides IIF/IID HCV EIA, although with much higher s/c ratios.

A much higher percentage of positive cases was found with those who have abnormal liver functions (24.7%) or previous infection(s) with Hepatitis B (30%

and 8%) when compared to those with other infections or diseases (e.g. 6.5%, 2.2% and 0%).

Note: Sera from HBsAg carriers were kindly provided by the Infectious Diseases Laboratory of the American Red Cross; sera from HBc antibody positive donors were obtained from Boston Biomedica Inc.; sera from individuals with elevated ALT levels (>100 I.U./L) were obtained from both Boston Biomedica Inc. and NABI laboratory; sera from asymptomatic individuals with retroviral antibodies (HIV-1 and HTLV-1) were obtained from New York Blood Center, and those with HIV-2 antibodies were from Guinea Bissau of West Africa, kindly provided by Dr. O. Varnier of Italy; sera from patients with ATL were kindly provided by the Japanese Red Cross; sera from patients with AIDS and ARC, were kindly provided by Dr. D. Knowles at Columbia University College of Physicians and Surgeons, and Dr. F. Siegal at the Long Island Jewish Hospital; sera from patients with various complications of autoimmune diseases were kindly provided by Dr. N. Chiorazzi of the Cornell University Medical School. All sera have been characterized by additional licensed serologic markers before inclusion in the current study.

Table 3 illustrates results obtained with the peptide based HCV EIA described in this invention on a panel of 23 recombinant HCV EIA repeatably reactive specimens obtained from a random donor population. Data on each specimen's ALT level and HBc antibody reactivity are provided as supplemental information for indirect confirmation of NANBH status of the positive donors. As can be seen from the Table, all eight specimens with indirect confirmation of their NANBH status scored positive in the peptide based EIA according to the present invention (on both IIG and IIF/IID plates). In addition, four specimens that scored high on the peptide based assay also scored as strong positives by the recombinant HCV EIA, thus further confirming the HCV positivity of these specimens. Only one specimen scored marginally positive on the peptide based HCV EIA, which lacks the other markers. However, this specimen scored positively with the recombinant HCV EIA. The remaining ten specimens that scored negative by the peptide based EIA according to the present invention all had a marginal s/cutoff ratio of between 0.9 to 2.6. FIG. 5 provides a direct correlation between the peptide based HCV EIA of the present invention and the recombinant based HCV EIA by their respective s/cutoff ratios for this panel. Thus, the peptide based HCV EIA of the present invention can clearly differentiate the repeatably reactive specimens

previously screened out by the rDNA based HCV EIA into two distinct groups, a positive group which correlated highly to those with other known NANBH markers and a negative group which probably represents specimens with extraneous reactivities unrelated to HCV. In addition to its use as a screening assay, the peptide based HCV EIA may also function as a positive confirmatory test for the rDNA based HCV EIA. Note: This well-characterized serum panel was kindly provided by Dr. C. Fang of the American Red Cross QC laboratory.

#### EXAMPLE 3

##### Detection of Antibodies to HCV By An Agglutination Based Assay

The presently claimed HCV peptides, synthesized according to the Merrifield solid phase method, can be conjugated to bovine serum albumin (BSA) by a simple crosslinking method in the presence of a low percentage of glutaraldehyde solution (0.025%), or with other crosslinking reagents such as m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) according to a previously published procedure (Biochemistry, 18:690-697, 1979). For example: to 0.32 mL of a BSA solution (10 mg/mL in 0.01M phosphate buffer, pH 7.0) at room temperature is added 0.013 mL of an MBS solution (0.025 mg/mL in dimethylformamide). The amount of MBS added to the BSA solution can be varied dependent on the optimal molar ratio of BSA to MBS determined for a specific conjugate studied. The mixture is stirred at room temperature for 1 hour, after which it is centrifuged to remove any precipitated albumin. The clarified mixture is then subjected to gel filtration on Sephadex G-25 and the protein-containing fractions, as detected by their absorbance at 280 nm, are pooled and stored frozen at  $-70^{\circ}\text{C}$ . until needed.

The peptides are dissolved in  $\text{H}_2\text{O}$  at 10 mg/mL. A predetermined amount of each peptide solution is added dropwise to the previously activated BSA-MBS solution and stirred at room temperature for 3 hours. The final peptide-BSA conjugates are separated from other free peptides by gel filtration or extensive dialysis. The ratio of peptide to BSA is determined by SDS-PAGE according to conventional methods.

Using the above mentioned peptide-BSA conjugation process, conjugated peptide IIG-BSA was absorbed to double aldehyde fixed human O erythrocytes at pH 4.0. The peptide-conjugate coated erythrocytes were then treated with  $\text{NaBH}_4$  to prevent non-specific protein binding. The peptide-conjugate coated erythrocytes were then washed with PBS and incubated with 5% normal human serum-PBS solution. These processed cells were then used in an agglutination assay for the detection of HCV antibodies in both serum and plasma specimens. The specimens were diluted 1:10 in a sample diluent buffer and an equal volume of the indicator cells (50  $\mu\text{L}$ ) was mixed with the diluted specimens. The agglutination pattern was settled within one hour; and the assay results were read by the naked eye and further quantitated by an optical device (manufactured by Olympus Corporation) which gave a P/C ratio, as determined by the absorbance readings of the periphery and center of the wells. In this experiment, a P/C ratio of 20 was set as the assay cutoff value, i.e. a positive agglutination pattern had a ratio of  $<20$  and a negative pattern,  $>20$ .

A total of 20 rDNA HCV EIA repeatedly reactive specimens were tested for antibodies to HCV in the

above-described HCV passive hemagglutination assay (PHA) employing Peptide IIG-BSA conjugate as the solid phase. FIG. 6 provides a correlation study between the peptide based HCV PHA and the recombinant based HCV EIA by their respective P/C and s/c ratios. All samples with s/c EIA ratios higher than 3 were found to be positive with the HCV PHA test. With the exception of one, all specimens having borderline s/c ratios (between 0.9 to 2) scored as negative in this PHA test.

#### EXAMPLE 4

##### Detection of Antibodies to HCV By An Agglutination Assay Utilizing As the Solid Phase Immunosorbent Gelatin Particles, Erythrocytes Of Different Animal Species, Or Latex Particles Coated with a Mixture of HCV Peptides

One mL thoroughly washed erythrocytes, gelatin particles, or polystyrene latex particles are coated with the HCV peptide mixture, or conjugates thereof at an effective concentration. The peptide mixture, or conjugates thereof, coated cells or particles are then incubated with serially diluted serum samples in the wells of a 96-well U-shaped microplate or on a slide. After being left at room temperature for about an hour, or a few minutes in the case of latex particle based microagglutination, the settled agglutination pattern on the bottom of each well or on the slide is read; and the highest dilution showing a positive reaction is recorded.

This is a one-step assay which can be used for both qualitative and quantitative detection of antibodies to HCV in specimens including sera or biofluids.

#### EXAMPLE 5

A test kit for detecting HCV antibodies using an agglutination assay comprises a compartmented enclosure containing multiple microwell plates and other accessory materials for an agglutination assay including (1) a bottle of HCV peptide coated erythrocytes, gelatin particles or latex polystyrene particles; (2) a negative control; and, (3) an inactivated HCV positive control, and (4) specimen diluent. The procedure described in Examples 3 and 4 is to be followed.

#### EXAMPLE 6

An enzyme immunoassay based diagnostic test kit for the detection of HCV antibodies can be constructed. The test kit comprises a compartmented enclosure containing multiple 96 well plates coated prior to use with the HCV peptide or peptide mixtures of the present invention in 100  $\mu\text{L}$  pH 9.5 10 mM  $\text{NaHCO}_3$  buffer. The kit further comprises materials for enzyme detection in separate sealed containers consisting of: 1) a negative control; 2) an inactivated HCV positive control; 3) specimen diluent; 4) peroxidase labeled-second antibody to human IgG; and 5) a color change indicator consisting of, for example, orthophenylenediamine (OPD) and hydrogen peroxide in a phosphate citrate buffer. The procedure described in Examples 1 and 2 is to be followed.

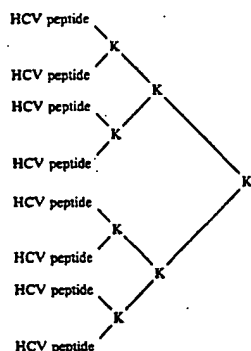
In this test kit, 96-well plates, precoated with a peptide or peptide mixture of the present invention, can be replaced by polystyrene beads, or multiple minicolumns filled with controlled pore size glass beads, or nitrocellulose paper strip, precoated with the peptides of the present invention for use as the solid phase immunosorbent.

## EXAMPLE 7

## Immunization with Octameric HCV Peptides for the Elicitation of Sustaining High Titers of HCV Antibodies

In addition to the use of synthetic HCV peptides as immunogens for the generation of sequence related anti-HCV antibodies for the ultimate development of an epitope-based subunit NANBH vaccine, another approach using a limited sequential propagation of a trifunctional amino acid lysine to form a core that serves as a low-molecular weight matrix carrier for peptide immunogens can also be applied. The trifunctional amino acid, Boc-Lys(Boc), is particularly suitable since both N- $\alpha$  and N- $\epsilon$  amino groups are available as reactive ends. Thus, sequential propagation of Boc-Lys(Boc) will generate 2<sup>n</sup> reactive ends. The first level coupling of Boc-Lys(Boc) will produce two reactive amino ends as a bivalent carrier. The sequential generations of a second and third step with Boc-Lys(Boc) will produce carriers containing four (tetra-valent), and eight (octa-valent) reactive amino ends to which peptide antigens are attached.

The HCV peptides as described in this invention can be incorporated onto this carrier system as illustrated below for the development of sustaining high titer HCV antibodies in mammals, including humans.



Octameric HCV peptides of the present invention (Table I) using the solid phase method of Merrifield are synthesized by an automated peptide synthesizer, either Applied Biosystems (ABI) Model 430A, or Biosearch Model 9500.

Both acid-labile tert-butyloxycarbonyl (t-Boc) and acid-stable groups are used for the protection of N- $\alpha$  amino acid and the functional side chains of the amino acids during the synthesis, respectively. The octameric peptides are synthesized by coupling onto a synthetic octamer resin.

An octamer resin is prepared by coupling di-t-Boc Lys onto 0.14 mmol/g MBHA (4-Methyl benzhydrylamine) resin. (Biosearch 9500 is used for this preparation due to its flexibility in scale). Di-Boc Lys single coupling is followed by two capping reactions (e.g. 0.3M Acetylimidazole in DMF dimethylformamide). The substitution level of synthetic octamer resin is determined by Ninhydrin Test.

Duncan Hartly random bred female guinea pigs (two per immunogen), weighing 400-500 gms, are used as the

hosts. For initial immunizations, an aliquot of 100  $\mu$ g octameric HCV Peptide in 0.5 mL PBS is mixed with an equal volume of complete Freund's adjuvant and injected into each animal both subcutaneously and intradermally over multiple sites. After two to three weeks of rest, an identical dosage of the same immunogen is given as a boost into each animal except that incomplete Freund's adjuvant is used. The animals are bled by heart puncture periodically to monitor each serum's anti-HCV titers. Subsequent booster shots are given periodically.

## EXAMPLE 8

## Relative (%) Immunoreactivity for Synthetic Peptides By An Enzyme-Linked Immunosorbent Assay

Wells of 96-well plates were coated at 4 $^{\circ}$  C. overnight (or 1 hour at 37 $^{\circ}$  C.), with each of the additional nine peptides, VA, VB, VC, VD, VE (=V), VIA, VIB, VIC, VID, VIE (see Table 1 for the above mentioned peptides), at 5  $\mu$ g/mL at 100  $\mu$ L per well in 10 mM NaHCO<sub>3</sub> buffer, pH 9.5. Each peptide's immunoreactivity was measured as previously described in Example 1. Results obtained for the 10 peptides in the V and VI series are shown in Table 2. According to the EIA absorbance readings at 492nm (y axis) and the amino acid sequences for each of the corresponding HCV peptides (x axis), representative immunoreactivity profiles are plotted for four of the eight sera on the 10 peptides in the V and VI series, together with the first twenty peptides in the I, II and III series, as shown in FIGS. 1-1 to 1-4. Relative (%) immunoreactivity index for each of the additional 10 peptides is likewise calculated using peptide IIID as a reference. Additional clusters of residues, such as ASRQA and EVIAP, that are identified with these 10 peptides, were found to contribute additionally to the overall HCV antibody reactivity.

In summary, epitope mapping analysis conducted with a series of overlapping peptides reveals a varying degree of immunoreactivities between different HCV antibody positive samples and these HCV peptides. Based on the above-mentioned epitope mapping study, a third representative EIA using peptides IIF, IIID and V as the solid phase antigen was also configured for testing as shown in Example 9 in comparison to that using peptide IIF and IIID.

## EXAMPLE 9

## Detection of Antibodies to HCV in Serial Samples by Enzyme-Linked Immunosorbent Assays

(a) A coded panel consisting of 24 samples derived from a case of transfusion transmitted NANBH were tested in two types of EIAs using plates coated with either a mixture of IIF and IIID at 5, 5  $\mu$ g/mL or a mixture of IIF, IIID and V at 5,5,5  $\mu$ g/mL. The panel was provided by Dr. H. Alter of NIH and the results were decoded by his laboratory.

As shown in FIG. 7-I, the two anti-HCV profiles, as tested by two formats, using Peptides IIF/IIID/V coated plate (Curve A) and Peptides IIF/IIID coated plate (curve B) respectively, spanning a ten year period revealed an interesting contrast.

According to the record, the seronegative patient received HCV contaminated blood units on Aug. 20, 1980. As a result of the transfusion, a trace amount of passive HCV antibodies was detected in the recipient's serum by format A. Active development of HCV antibodies by the recipient became detectable by both for-

mats from November 14th on (about three months after the initial transfusion). The HCV antibodies, developed as a result of HCV infection through blood transfusion, persisted throughout the next ten years. Higher antibody signals were detected by plates coated with an extra peptide V (curve A) in sera collected four months after the transfusion. It appears that the epitope presented by peptide V, representing a neighboring immunodominant region, elicits abundant HCV antibodies at a slightly later stage than the epitopes represented by peptides IIF and IIID.

(b) Serial samples from one representative case of a hemodialysis patient with NANBH were provided by Dr. Cladd Stevens of New York Blood Center, N.Y., N.Y., and tested on plates coated with a mixture of three peptides, IIF/IIID/V. The sample histories are shown in FIG. 7-2. The results show that the peptide based EIA detects samples about two months after the onset of the acute phase of the disease as evidenced by the ALT elevation.

(c) Serial samples from a representative chimpanzee were tested with a peptide based HCV EIA using a mixture of IIF/IIID/V peptides. This chimpanzee was inoculated on day 0 with a well-characterized strain of NANBH. Following the acute phase of infection as evidenced by the rise of the ALT levels, antibodies to HCV were detected about 60 days after inoculation [FIG. 7-3].

#### EXAMPLE 10

##### Screening of Low Risk Random Blood Donors With the Peptide Based HCV EIA

2035 donor specimens obtained in a blood bank setting were tested by EIA coated with a mixture of Peptides IIF, IIID and V at 5 ug/mL each following the procedure described in Example 2. The results are shown in FIGS. 8-1 and 8-2. The frequency distribution of the peptide based HCV-EIA signal to cutoff ratios, suggested an initial reactive rate of 1.18% and a repeatably reactive rate of 1.08 respectively. 88% of the initial reactive specimens are repeatably reactive indicating a high reproducibility of the assay. The repeatably reactive rate of the peptide based HCV EIA obtained with the low risk random blood donor specimens, all volunteers, is lower than that obtained from the commercial paid donor population (See Example 2).

#### EXAMPLE 11

##### Synthetic Peptide Based HCV Neutralization EIA As a Confirmatory Test

Wells of 96-well plates were coated at 4° C. overnight (or for 1 hour at 37° C.) with a mixture of two peptides IIF and IIID at 5 ug/mL each in 100 uL 10 mM NaHCO<sub>3</sub> buffer pH 9.5. Repeatably reactive specimens previously screened out by the direct HCV EIA were incubated with either a control specimen diluent buffer (i.e., PBS containing 20% by volume normal goat serum, 1% by weight gelatin and 0.05% by volume Tween 20) at a dilution of 1:20 volume to volume, or with the same specimen diluent buffer containing varying amounts of a HCV peptide analogue IV (see Table 1 for its amino acid sequence) and allowed to react for an hour at 37°.

200 uL of the peptide IV neutralized specimens were then added to each of the wells and allowed to react for 15 minutes at 37°, followed by the EIA procedure as

described in Example 2. Four representative reactive samples including two weakly reactives and two strongly reactives were tested. One of the strong reactives was further diluted at 1:10 in the specimen diluent prior to neutralization testing. As shown in FIG. 9 and Table 4, a dose dependent inhibition [or neutralization] of HCV EIA was observed with peptide IV. When compared with the controls, a significant inhibition was obtained with all four specimens even at a concentration of 50 ug/mL peptide IV.

#### EXAMPLE 12

##### Detection of Antibodies to HCV in Hemodialysis patients by EIA

A coded panel consisting of 74 samples from a group of hemodialysis patients was tested in two types of EIAs using plates coated with a mixture of HCV peptides IIF and V at 10.5 ug/mL or a recombinant HCV protein based EIA. The panel was provided by investigators at the Japanese National Institute of Health and the results were decoded and compared to the recombinant HCV protein based EIA by the sera provider.

As shown in FIG. 10, an x-y plot of the A492 nm readings for the peptide based HCV EIA and the recombinant HCV protein based HCV EIA revealed a high correlation between these two assays. (A cutoff value of 0.2 and 0.4 was obtained based on the corresponding assay design.) These 74 specimens obtained from the hemodialysis patients who are highly susceptible to HCV infection were grouped into four categories based on their respective reactivities with these two types of EIAs. The upper right block indicates samples that are scored positive by both assays, and the lower left block indicates samples that are scored negative by both assays. None of the 74 high risk samples were found positive by the recombinant based EIA and negative by the peptide based EIA as shown in the upper left block; whereas five of these 74 high risk samples scored positive by the peptide based EIA and negative by the recombinant based EIA as shown in the lower right block indicating that the peptide based HCV EIA is more sensitive when tested with specimens derived from patients at high risks for HCV infection.

#### EXAMPLE 13

##### Detection Of Anti-HCV Activity In Rare Specimens With An Elevated ALT Level

These results are representative of the acute phase of HCV infection by synthetic peptides of Peptide VII series, covering a region near the C-terminus of the HCV protein C-100, and Peptide IIF from the immunodominant region.

Wells of 96-well plates were coated at 37° C. for 1 hour with each of the six peptides VIIA, VIIB, VIIC, VIID, IIG and IIF, at 5 ug/mL at 100 uL per well in 10 mM NaHCO<sub>3</sub> buffer, pH 9.5. Each peptide's immunoreactivity with the respective specimen was measured as previously described in Example 1. As shown in Table 5, weak immunoreactivity was obtained with specimen 3 for peptides VIIC and VIID, but not VIIA and VIIB. Moderate immunoreactivity was obtained with specimen NAB-2-2 for peptide IIF, but not IIG. Both specimens were found to have high ALT level and are representative of specimens from patients with acute phase of HCV infection.

TABLE 4

Peptide Based HCV Neutralization EIA As A Confirmatory Test											
Specimens		Peptide IV Concentration									
		400 ug/mL		200 ug/mL		100 ug/mL		50 ug/mL		Control	
ID	Dilution	mA	% I	mA	% I	mA	% I	mA	% I	mA	% I
A	1:1	590	72.0	1129	46.5	1066	49.5	1363	35.4	2111	
	1:10	244	86.1	325	81.5	409	76.7	510	71.0	1762	
B	1:1	161	92.0	209	89.6	321	84.1	523	74.1	2021	
	1:1	117	89.0	162	84.7	155	85.4	153	85.6	1064	
D	1:1	27	89.8	23	91.3	38	85.7	34	87.2	266	
	1:1										

% INHIBITION =	$\frac{\text{mA}(\text{control}) - \text{mA}(\text{ug/mL})}{\text{mA}(\text{control})}$
----------------	---

$$\% \text{ INHIBITION} = \frac{\text{mA}(\text{control}) - \text{mA}(\text{ug/mL})}{\text{mA}(\text{control})}$$

TABLE 5

Code	Amino Acid Sequence	A492 nm	
		Specimen No.	
IIH	SG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL	1.332(+)	
IIG	II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMMLA, EQFKQ, KALGL	0.013(-)	
VIIA	AVQWM, NRLIA, FASRG, NHVSP	0.109	
VIIIB	RHV, GPGE, AVQWM, NRLIA, FASRG, NHVSP	0.224	
VIIIC	V, VCAAL, LRRHV, GPGE, AVQWM, NRLIA, FASRG, NHVSP	0.674	
VIIID	PGA, LVVGV, VCAAL, LRRHV, GPGE, AVQWM, NRLIA, FASRG, NHVSP	0.658	

## EXAMPLE 14

Measurement Of Relative (%) Immunoreactivity For Synthetic Peptide Covering An Immunodominant Region Of The Postulated HCV Core Protein By An Enzyme-Linked Immunosorbent Assay

Wells of 96-well plates were coated at 4° C. overnight (or 1 hour at 37° C.), with each of the ten peptides: VIIIA, VIIIB, VIIIC, VIID, VIIIE(=VIII), IXA, IXB, IXC, IXD, and IXE(=IX), (see Table 7) prepared as described at 5 ug/mL at 100 uL per well in 10 mL NaHCO<sub>3</sub> buffer, pH 9.5. The rest of the plate coating and enzyme immunoassay procedures were performed exactly as described in Example 1.

Results obtained from this study are shown in FIGS. 11-1 and 11-2. According to the EIA absorbance readings at 492 nm (y axis) and the amino acid sequences for each of the corresponding HCV peptides (x axis), representative immunoreactivity profiles are plotted for four of the eight panel sera as shown in FIGS. 11-1 and 11-2. Relative (%) immunoreactivity index for each of the 10 peptides (Table 7) is calculated against Peptide IIID (See Table 1), the one with the highest absorbance reading, based on the total absorbance of eight sera at 492 nm (See Tables 1 and 2 for examples of calculation) FIGS. 11-1 and 11-2 show the amino acid sequences of the immunodominant region according to data obtained for immunoreactivity study. For example, serum sample 1 reacted strongly (ODs between 1.5 and 3.5) with peptides VIIIA and IXA, which are the smallest size in the 20 mer range in the corresponding peptide series. Further addition of amino acids at the N-terminal end did not significantly enhance the immunoreactivity of these analogue peptide (see FIGS. 11-1 and 11-2 for sample 1).

However, other serum samples such as #3 and #4 reacted much stronger with peptides VIIIB, VIIIC respectively and with an increasing immunoreactivity with the analogue peptides in the IX series (see FIGS. 11-1 and 11-2, samples #3 and #4). Further, serum sample 2 reacted marginally with peptides in the corresponding VIII and IX series. These reactivity profiles indicate a more complicated epitope distribution along the postulated HCV core protein region and may in-

clude some discontinuous linear epitopes and conformational epitopes, requiring a longer size peptide to confer the best immunoreactivity for diagnostic purposes.

In summary, epitope mapping analysis conducted with a series of ten peptides covering an immunodominant region of the postulated HCV core protein, spanning a total of 119 amino acid residues as illustrated in Table 7 and FIGS. 11-1 and 11-2, reveals varying degrees of immunoreactivity between different HCV antibody positive serum samples and analogue HCV peptides of the VIII and IX series. In this case it is found preferably to have synthetic peptides with longer amino acid chains, ideally longer than 20, to optimally present these antigenic determinants to HCV antibodies.

Based on the above-mentioned epitope mapping study, additional representative EIAs using Peptides VIIIE, IXD, both derived from the HCV core region alone, or as a mixture with peptides IIH and V from the HCV nonstructural region were configured for the following studies described in Examples 15, 16, 17 and 18.

## EXAMPLE 15

Detection Of Antibodies To HCV By Peptide Based Enzyme-Linked Immunosorbent Assay Using Format C. Format D. Format A

The following four groups of specimens:

- individuals with AIDS, ARC(n=63);
  - individuals positive for HBsAg, (n=50);
  - individuals positive for antibodies to HBe protein, (n=22); and
  - individuals with elevated (>100 i.u./L) alanine aminotransferase (ALT) enzyme activity, (n=86).
- were analyzed on representative HCV peptide based EIAs according to the present invention, with the plates coated either with (i) peptides IIH and V at 5 and 3 ug/mL each (Format A), (ii) peptides IIH, V and VIIIE at 5, 3 and 2 ug/mL each (Format C, containing both the HCV core and nonstructural peptides) or (iii) Peptides VIIIE and IXD at 2 and 2 ug/mL each (Format D, HCV core peptides only).

Results obtained from the screening of a total of 221 well-characterized clinical specimens previously categorized into four groups, from (a) to (d) using a representative lot of peptide coated plates EIAs formatted as A, C or D were plotted on histograms as shown in FIGS. 12-1, 12-2 and 12-3.

Out of a total of 63 AIDS/ARC patient samples analyzed, 46.0%, 55.6% and 50.8% of the patients were found to be HCV antibodies positive using EIA formats A, C and D respectively. Out of 50 HBsAg positive individuals, 36.0%, 42.0% and 36% of the individuals were found to also be HCV antibodies positive using EIA formats A, C and D respectively. Out of 22 HBe antibody positive individuals, 27.3%, 22.7%, and 18.2% were found to be HCV antibodies positive as detected by EIA formats A, C and D. Out of 86 patients with an elevated ALT levels, 90.7%, 91.5% and 85.4% were found to be HCV antibodies positive by EIA formats A, C and D. The overall signal to noise ratio distribution for the HCV positive samples were found to be higher with Formats C and D which included a peptide (VIIE) from the HCV core region than Format A which only employed peptides from the HCV nonstructural region as the solid phase antigen.

Except for one HBe antibody sample where the results is borderline positive (S/cutoff ratio ~ 1.0) with the HCV EIA Format A, Format C incorporating peptides (IIH, V and VIIIE) from both the HCV structural (core) and nonstructural regions was the most sensitive. The significant improvement in sensitivity makes Format C an ideal candidate for a HCV antibody screening assay.

#### EXAMPLE 16

##### Comparison Of Test Results Using The Three Peptide Based HCV EIA Formats (A,C And D) On Low Risk Random Blood Donors

Representative 264 donor specimens obtained in a blood bank setting were tested by all three EIA formats.

The results are shown in FIGS. 13-1 to 13-6. The frequency distributions of the peptide based HCV-EIA signal to cutoff ratios suggested an initial reactive rate of 1.13%, 3.0% and 3.0% with formats A, C and D respectively. The negative samples have a relative low signal to cutoff ratio in all three assay formats (see FIGS. 13-1, 13-3, and 13-5). Upon repeat testing, a repeatably reactive rate of 1.13%, 1.9% and 1.9% were obtained for formats A, C and D respectively. Among the sera identified as positives, there were four specimens which reacted strongly with both Formats C and D, but were identified as negatives by Format A. This indicates the possibility of false negative results when an HCV antibody detection assay does not include epitopes from the structural protein region.

#### EXAMPLE 17

##### Detection Of Antibodies To HCV In Well-Characterized Serial Samples By Various Enzyme-Linked Immunosorbent Assays

(a) A coded panel consisting of 24 samples derived from a case of transfusion transmitted NANBH were tested in three HCV EIA formats (A, B and C) to determine the respective sensitivity of the formats in detecting seroconversion. The panel was provided by Dr. H. Alter of NIH and the results were decoded by his laboratory.

As shown in FIG. 14-1, the three anti-HCV profiles, as tested by three formats using peptides IIF/IIID/V

coated plate (Curve A); Peptides IIF/IIID coated plate (Curve B); and peptides IIH, V and VIIIE coated plate (Curve C) respectively, with sera spanning a ten year period revealed an interesting contrast.

As a result of the transfusion, a trace amount of passive HCV antibodies was detected in the recipient's serum by both format A, and C. Active development of HCV antibodies by the recipient became detectable by all three formats from November 14th on (about three months after the initial transfusion), with format C having the highest S/cutoff ratio on that bleed date. This finding further confirms the improved sensitivity obtained by using HCV EIA format C.

(b) Serial samples from a representative chimpanzee were tested with HCV EIA format C in comparison to a recombinant HCV C-100 based radioimmunoassay (RIA). The chimpanzee was inoculated on day 0 with a well-characterized strain of NANBH. Following the acute phase of infection as evidenced by the rise of the ALT levels, antibodies to HCV were detected about 47 days after inoculation (FIG. 14-2). HCV EIA Format C was able to detect HCV antibodies about 40 days early than the rDNA based RIA. A higher signal to cutoff ratio was obtained with HCV EIA Format C than the rDNA RIA.

(c) Serial samples from three well-characterized representative HCV seroconversion panels, collected by Serologic Inc., were tested by HCV EIA formats A, C and D, as defined in Example 15 in addition to that previously tested with rDNA HCV C-100 based EIA. As shown in Table 8, both HCV EIA formats C and D were able to identify HCV antibody positive specimens in two out of three panels by four to eight weeks earlier than the rDNA HCV-100 based EIA and HCV EIA Format A. This further demonstrated the sensitivity of the HCV EIAs which incorporate peptides derived from the HCV structural (core) protein region.

#### EXAMPLE 18

##### Detection Of Antibodies To HCV In Hemodialysis Patients By Various Forms Of HCV EIAs

A coded panel consisting of 74 samples from a group of hemodialysis patients was tested by three types of HCV EIAs; a recombinant HCV protein based EIA, and two using plates coated with either a mixture of HCV peptides IIH and V at 10, 5 ug/mL respectively (Format A), or a mixture of HCV peptides IIH, V and VIIIE at 5, 3 and 2 ug/mL respectively (Format C). The panel was provided by investigators at the Japanese National Institute of Health. Results were decoded and compared to the recombinant HCV protein based EIA by the sera provider.

As shown in FIG. 15-1, an x-y plot of the A492 nm readings for the peptide based HCV EIA Format C and the recombinant HCV protein based HCV EIA revealed an increased sensitivity with the peptide based HCV EIA format C when compared to the rDNA HCV C-100 protein based HCV EIA. (A cutoff value of 0.2 and 0.4 was obtained based on the corresponding assay design). These 74 specimens obtained from dialysis patients who are highly susceptible to HCV infection were grouped into four categories based on their respective reactivities with these two types of EIAs. The upper right block indicates samples that were scored positive by both assays, and the lower left block indicates samples that were scored negative by both assays.

None of the 74 high risk samples were found positive by the recombinant based EIA and negative by the peptide based EIA as shown in the upper left block; whereas "eleven" of these 74 high risk samples scored positive by the peptide based EIA Format C and negative by the recombinant based EIA as shown in the lower right block.

An increase in sensitivity was obtained for the peptide based HCV EIA Format C (incorporating a HCV core peptide) when compared to HCV EIA Format A, which in turn showed an improved sensitivity compared with the recombinant HCV C-100 protein based EIA (see Example 12, FIG. 10).

To further document the validity of such a sensitivity comparison, other clinical data obtained for each of the dialysis patient specimens were tabulated along with the corresponding EIA ratios (Table 9). Among the eleven

marked specimens, most showed an increased level of GOT/GPT and were associated with frequent episodes of elevated GPT previously. All eleven specimens scored negative by the rDNA HCV C-100 based EIA. However, these same samples reacted strongly (with O.D. ~1.5) in the peptide based HCV EIA Format C. Since peptide VIII(=VIIIe) was synthesized according to amino acid sequences selected from the conserved structural (core) protein region, its inclusion in the peptide based HCV EIA (such as format C) will be particularly suitable when testing specimens from geographically distinct regions where a higher chance of strain-to-strain variation among the HCV isolates may be encountered.

It is to be understood that the above examples are illustrative of the present invention and are not meant to limit the scope thereof.

TABLE 8

Testing of Various Formats of HCV EIAs with Three Well-Characterized Seroconversion Panels									
Panel	Donor #	Bleed Date	ALT I.U./L	AST I.U./L	rDNA HCV c-100	EIA Ratio			HCV EIA Format D (core)
						HCV EIA Format A (ns)	HCV EIA Format C (core + ns)	HCV EIA Format D (core)	
Panel 1 02190D	880809		40.0	NA	0.03	0.093	0.108	0.205	
	880816		32.0	NA	0.04	-0.014	0.045	0.129	
	880823		32.0	NA	0.06	-0.050	0.025	0.072	
	880830*		180.0	121.0	0.04	-0.050	1.037*	1.096*	
	880928		401.0	352.0	0.19	0.100	7.193	7.703	
	881109*		NA	NA	6.57*	16.700*	10.185	7.281	
Panel 2 00269B	881122		NA	NA	6.57	16.671	9.770	9.321	
	880815		39.0	NA	0.0	0.014	-0.058	-0.008	
	880825		274.0	310.0	0.0	0.443	0.058	0.108	
	880829		346.0	270.0	0.0	0.029	0.128	0.185	
	880914		1175.0	722.7	6.5*	4.057	7.835*	5.984*	
	881005		429.7	172.3	6.5	5.857	7.811	5.851	
Panel 3 20830D	880829		63.0	65.0	-0.04	-0.043	0.115	0.181	
	880901*		81.0	NA	0.04	0.043	1.607*	1.108*	
	880908		183.0	174.0	0.02	-0.043	2.506	3.116	
	880928*		563.0	553.0	6.57*	3.800	9.827	9.659	
	881026		436.0	151.0	6.57	13.786	10.630	10.566	

TABLE 9

HCV Positivity in Serum Specimens Obtained from Japanese Dialysis Patients							
Code No.	rDNA based HCA EIA OD Cutoff = 0.40	Peptide based HCV EIA Format A Cutoff = 0.205	Peptide based HCV EIA Format C Cutoff = 0.204	HBsAb	GOT/GPT Oct., 89	n: times during 1986-1988 when GPT >25 I.U./L	
24	0.038	-0.001	0.005		2/3	0	
25	0.042	0.005	0.007		9/9	0	
26	0.105	-0.001	-0.003		4/4	0	
27	1.837	1.469	2.312	-	3/6	2	
28	1.797	1.637	2.398	-	20/21	2	
29*	0.011	0.001	1.603		7/4	0	
30	0.994	0.374	2.213		11/9	0	
31	1.823	0.425	0.874	-	27/16	4	
32	0.770	0.372	0.500	+	17/7	9	
33	1.712	2.101	2.234	-	28/32	29	
34	0.002	-0.003	0.007		11/14	0	
35*	0.026	0.161	2.229	+	14/23	23	
36*	0.065	0.018	2.286		20/18		
37	0.021	0.000	0.011	+	16/11	1	
38	2.347	1.917	2.182	+	26/23	6	
39	0.008	-0.007	0.004		7/6	0	
40	0.026	0.006	-0.002		10/8	0	
41*	0.061	0.118	1.933	+	9/6		
42	2.481	2.144	2.211	-	13/19	2	
43	0.008	-0.005	-0.005	+	11/7		
44	0.009	-0.004	-0.005		4/4	0	
45	0.009	0.000	-0.003		7/2	0	
46	2.177	1.990	2.121	-	16/12	8	
47	0.023	0.003	0.015		7/3	0	
48	0.025	-0.003	0.002	+	18/11		



TABLE 9-continued

HCV Positivity in Serum Specimens Obtained from Japanese Dialysis Patients						
Code No.	rDNA based HCA EIA OD Cutoff = 0.40	Peptide based HCV EIA Format A Cutoff = 0.205	Peptide based HCV EIA Format C Cutoff = 0.204	HBsAb	GOT/GPT Oct., 89	n: times during 1986-1988 when GPT >25 I.U./L
49	0.025	-0.001	-0.006		9/3	0
50	0.026	0.024	-0.003		9/3	
51	0.018	-0.003	-0.007	+	11/5	
52*	0.011	-0.003	1.366	-	33/52	29
53	2.251	1.296	2.218		8/7	0
54	0.050	0.017	0.040		10/7	0
55	0.020	-0.007	0.017	+	14/8	
56	0.033	-0.004	0.000		9/3	0
57	1.396	0.718	2.121	-	17/11	1
58	0.045	0.013	-0.003		13/12	
59	0.014	0.068	0.056		10/7	0
60	0.009	0.014	0.056	+	15/0	10
61	2.007	2.214	2.235	+	12/9	
62	0.171	0.001	0.003		11/7	0
63	1.121	0.529	2.383	+	18/10	
64	0.113	0.066	0.002		4/3	0
65	0.032	0.003	-0.003	+	7/5	3
66	0.036	-0.001	-0.002	+	11/6	
67*	0.049	0.037	2.119		16/11	
68*	0.177	0.638	2.000	+	24/25	33
69	0.027	0.007	-0.007		6/3	0
70	0.031	-0.006	-0.001		16/9	0
71	0.781	0.473	2.151	+	13/8	14
72	0.110	0.002	0.059		13/8	0
73	0.043	-0.002	-0.007	-	2/3	0
74	0.014	0.001	-0.004		2/3	0
75	0.053	0.000	0.019	+	15/8	
76	0.060	0.015	0.018		14/7	0
77	0.011	0.001	-0.004		8/8	
78	0.042	0.002	0.023		3/0	0
79	0.537	0.219	1.742	+	11/7	
80	2.615	1.713	2.428	+	18/16	12
81	2.509	2.265	2.294		9/4	
82	0.019	0.000	0.120		11/5	0
83	0.511	1.928	2.229	-	19/11	5
84	0.020	0.016	0.095	-	12/9	
85	0.013	-0.003	0.116		10/7	0
86	0.003	-0.005	-0.006		19/5	
87	0.031	-0.009	0.009		10/6	0
88	0.039	0.019	0.004		6/2	0
89*	0.273	0.223	2.055	-	10/8	8
90	0.045	0.026	-0.002	-	7/3	3
91	0.018	0.003	-0.002		5/8	0
92	1.974	1.127	2.189	+	11/23	22
93	0.893	1.113	2.226	-	24/19	5
94*	0.267	0.353	2.029	-	18/12	7
95	0.026	-0.010	0.000	-	34/73	0
96*	0.021	0.002	1.599	-	13/30	27
97*	0.246	0.037	1.779		15/9	0
98	2.412	1.904	2.236	-	3/9	

We claim:

1. A peptide composition comprising a peptide selected from the group consisting of Peptide I, III to IX, each peptide with an amino acid sequence as follows:

Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-  
Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-  
Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-  
Pro-X  
(Peptide I)

Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-  
Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-  
Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-  
Ile-X  
(Peptide III)

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-

-continued

Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-  
Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-  
Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-  
Phe-X  
(Peptide IV)

Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-  
Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-  
Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-  
Phe-X  
(Peptide V)

Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-  
Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-  
Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-  
Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-  
Glu-Thr-X  
(Peptide VI)

55

(i)

(iv)

60

(ii)

(v)

65

(iii)

-continued

Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-  
 Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-  
 Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-  
 Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-  
 His-Val-Ser-Pro-X  
 (Peptide VII)

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-  
 Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-  
 Asp-Val-Lys-Phe-Pro-Gly-Gly-Gln-Ile-  
 Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-  
 Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-  
 Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-  
 Arg-Gly-Arg-Arg-X  
 (Peptide VIII)

and

Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-  
 Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-Gln-Pro-  
 Gly-Tyr-Pro-Trp-Pro-Leu-Thr-Gly-Asn-Glu-  
 Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-  
 Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-  
 Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-  
 Leu-Gly-X  
 (Peptide IX)

wherein X is —OH or —NH<sub>2</sub>; and

- (ix) a. an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved;
- b. a segment of each of the above peptides or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 17.8% up to 101%;
- c. a mixture of the above peptides or analogues of the peptides;
- d. a conjugate of each of the peptides with carrier proteins, the conjugate having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 17.8% up to 101%; and
- e. a polymer of each of the peptides comprising a branching dimer, tetramer, or octamer of the peptide on a mono, tri, or hepta lysine core respectively.

2. A peptide composition comprising Peptide II having an amino acid sequence as follows:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
 Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
 Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
 Glu-Glu-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
 Lys-Gln-Lys-Ala-Leu-Gly-Leu-X  
 (Peptide II)

and an analogue thereof having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to said peptide and having specific immunoreactivity to HCV relative to said peptide that is substantially preserved.

3. A peptide composition according to claim 2 wherein the peptide comprises a segment of Peptide II and has an amino acid sequence selected from the group consisting of:

Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-  
 Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-  
 Leu-Gly-Leu-X;  
 (Peptide IIC)

-continued

(vi)

Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-  
 Tyr-Ile-Glu-Glu-Gly-Met-Met-Leu-Ala-Glu-Gln-  
 Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
 (Peptide IID)

(vii)

Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-  
 Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-  
 Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-  
 Leu-Gly-Leu-X;  
 (Peptide IIE)

(iv)

Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-  
 Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-  
 Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
 Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
 (Peptide IIF)

(viii)

20 wherein X is —OH or —NH<sub>2</sub> and an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

4. A peptide composition according to claim 1 wherein the peptide comprises a segment of Peptide III and has an amino acid sequence selected from the group consisting of:

Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-  
 Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-  
 Pro-Tyr-Ile-X;  
 (Peptide IIIC)

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-  
 Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-  
 Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;  
 (Peptide IIID)

Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-  
 Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-  
 Asp-Glu-Met-Gln-Glu-Cys-Ser-Gln-His-Leu-Pro-  
 Tyr-Ile-X;  
 (Peptide IIIE)

wherein X is —OH or —NH<sub>2</sub> and an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

5. A peptide composition according to claim 4 wherein the peptide has an amino acid sequence as follows:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-  
 Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-  
 Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;  
 (Peptide IIID)

wherein X is —OH or —NH<sub>2</sub> or an analogue of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

6. A peptide composition according to claim 1 wherein the peptide comprises a segment of Peptide VIII and has an amino acid sequence selected from the group consisting of:

(i)  
Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIII D)

(ii)  
Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIII C)

(iii)  
Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIII B)

(iv)  
Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIII A)

wherein X is —OH or —NH<sub>2</sub> or an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

7. A peptide composition according to claim 1 wherein the peptide comprising a segment of Peptide IX and has an amino acid sequence selected from the group consisting of:

(i)  
Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;  
(Peptide IX D)

(ii)  
Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;  
(Peptide IX C)

(iii)  
Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;  
(Peptide IX B)

(iv)  
Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;  
(Peptide IX A)

wherein X is —OH or —NH<sub>2</sub> or an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunore-

activity to antibodies to HCV relative to the peptide that is substantially preserved.

8. A peptide composition according to claim 1 wherein the peptide has an amino acid sequence as follows:

Ser-Thr-Pro-Lys-Pro-Gln-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X

wherein X is —OH or —NH<sub>2</sub>, or an analogue of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide, and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved; and a segment of the above peptide having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 17.8% up to 101%.

9. A peptide composition according to claim 1 wherein the peptide has an amino acid sequence as follows:

Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is —OH or —NH<sub>2</sub>, an analogue of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide, and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved; and a segment of the above peptide or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 17.8% up to 101%.

10. An enzyme linked immunosorbent assay (ELISA) test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

- (i) a solid substrate coated with a peptide composition according to claim 1;
- (ii) a negative control sample;
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.

11. An ELISA test kit according to claim 28 wherein the solid phase is coated with a peptide composition comprising a segment of Peptide II and having an amino acid sequence selected from the group consisting of:

(i)  
Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

(ii)  
Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

(iii)  
Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-

-continued

Lys-Ala-Leu-Gly-Leu-X;

- (iv) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-  
Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-  
Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-  
Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-  
X;
- (v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

wherein X is —OH or —NH<sub>2</sub>, and an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

12. An ELISA test kit according to claim 10 wherein solid phase is coated with a peptide having an amino acid sequence:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

wherein X is OH or —NH<sub>2</sub> and an analogue of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

13. An ELISA test kit according to claim 10 wherein the solid phase is coated with a peptide composition comprising a segment of Peptide III having an amino acid sequence selected from the group consisting of:

- (i) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-  
Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-  
His-Leu-Pro-Tyr-Ile-X;
- (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;
- (iii) Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-  
Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-  
Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-  
His-Leu-Pro-Tyr-Ile-X;

wherein X is —OH or —NH<sub>2</sub> and an analogue of each of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

14. An ELISA test kit according to claim 13 wherein the peptide is:

- (i) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

wherein X is —OH or —NH<sub>2</sub> or an analogue of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to

antibodies to HCV relative to the peptide that is substantially preserved.

15. An ELISA test kit according to claim 10 wherein solid phase is coated with a peptide composition comprising a segment of Peptide VIII having an amino acid sequence selected from the group consisting of:

- (i) Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-  
Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-  
Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-  
Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-  
Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-  
Arg-Arg-X;
- (ii) Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-  
Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-  
Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-  
Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-  
Arg-Arg-X;
- (iii) Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-  
Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-  
Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-  
Arg-Arg-X;
- (iv) Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-  
Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-  
Arg-Arg-X;

wherein X is —OH or —NH<sub>2</sub> or an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

16. An ELISA test kit according to claim 10 wherein solid phase is coated with a peptide composition comprising a segment of Peptide IX having an amino acid sequence selected from the group consisting of:

- (i) Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-  
Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-  
Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-  
Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-  
Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-  
Arg-Ser-Arg-Asn-Leu-Gly-X;
- (ii) Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-  
Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-  
Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-  
Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-  
Arg-Ser-Arg-Asn-Leu-Gly-X;
- (iii) Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-  
Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-  
Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-  
Arg-Ser-Arg-Asn-Leu-Gly-X;
- (iv) Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-  
Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-  
Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is —OH or —NH<sub>2</sub> or an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

17. An ELISA test kit according to claim 10 wherein the solid phase is coated with a peptide composition comprising a peptide having an amino acid sequence as follows:

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-  
Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-

-continued

Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-  
Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-  
Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-  
Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-  
Arg-X

wherein X is —OH or —NH<sub>2</sub> or an analogue of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide, and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved; and a segment of the above peptide or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 17.8% up to 101%.

18. An ELISA test kit according to claim 10 wherein the solid phase is coated with a peptide composition comprising a peptide having an amino acid sequence as follows:

Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-  
Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-  
Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-  
Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-  
Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-  
Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-  
Gly-X;

wherein X is —OH or —NH<sub>2</sub> and an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

19. An ELISA test kit according to claim 10 wherein the solid phase is coated with a peptide composition comprising a mixture of Peptides IIH and V, Peptides IIH and V having the following amino acid sequences respectively:

(i) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-  
Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-  
Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-  
His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-  
Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-  
Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)

(ii) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-  
Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-  
Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-  
Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-  
Trp-Ala-Lys-His-Met-Trp-Asn-Phe-  
X; (V)

wherein X is —OH or —NH<sub>2</sub> or an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

20. An ELISA test kit according to claim 10 wherein the solid phase is coated with a peptide composition comprising a mixture of Peptides IIF, IIID and V, Peptide IIF, IIID and V having the following amino acid sequences respectively:

(i) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Gln-  
Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-  
His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-  
Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-  
(IIF)

-continued

Ala-Leu-Gly-Leu-X;

(ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-  
Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-  
Tyr-Ile-X; (IIID)

(iii) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-  
Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-  
Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-  
Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-  
Met-Trp-Asn-Phe-X; (V)

wherein X is —OH or —NH<sub>2</sub> or an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

21. An ELISA test kit according to claim 10 wherein the solid phase is coated with a peptide composition comprising a mixture of Peptides IIH, V and VIIIE, Peptide IIH, V and VIIIE having the following amino acid sequences respectively:

(i) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-  
Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-  
Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-  
Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-  
Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-  
Gly-Leu-X; (IIH)

(ii) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-  
Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-  
Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-  
Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-  
Met-Trp-Asn-Phe-X; (V)

(iii) Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-  
Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-  
Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-  
Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-  
Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-  
Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-  
Ser-Gln-Pro-Arg-Gly-Arg-Arg-X; (VIIIE)

wherein X is —OH or —NH<sub>2</sub> or an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

22. An ELISA test kit according to claim 10 wherein the solid phase is coated with a peptide composition comprising a mixture of Peptides VIIIE and IXD, Peptide VIIIE and IXD having the following amino acid sequences respectively:

(i) Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-  
Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-  
Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-  
Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-  
Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-  
Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-  
Ser-Gln-Pro-Arg-Gly-Arg-Arg-X; (VIIIE)

-continued

(ii) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is —OH or —NH<sub>2</sub> and an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide, and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved; a segment of each of the above peptide or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 17.8% up to 101%.

23. A peptide having the amino acid sequence:

(Peptide II)  
Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X

wherein X is —OH or —NH<sub>2</sub>.

24. A peptide having the amino acid sequence:

(Peptide III)  
Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X

wherein X is —OH or —NH<sub>2</sub>.

25. A peptide having the amino acid sequence:

(Peptide IV)  
Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X

wherein X is —OH or —NH<sub>2</sub>.

26. A peptide having the amino acid sequence:

(Peptide V)  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Am-Phe-X

wherein X is —OH or —NH<sub>2</sub>.

27. A peptide having the amino acid sequence:

(Peptide VI)  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X

wherein X is —OH or —NH<sub>2</sub>.

28. A peptide having the amino acid sequence:

(Peptide VII)  
Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-

-continued

(IXD) Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X

wherein X is —OH or —NH<sub>2</sub>.

29. A peptide having the amino acid sequence:

(Peptide VIII)  
Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X

wherein X is —OH or —NH<sub>2</sub>.

30. A peptide having the amino acid sequence:

(Peptide IX)  
Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

wherein X is —OH or —NH<sub>2</sub>.

31. A peptide having the amino acid sequence:

(Peptide IIF)  
Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X

wherein X is —OH or —NH<sub>2</sub>.

32. A peptide having the amino acid sequence:

(Peptide IIID)  
Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X

wherein X is —OH or —NH<sub>2</sub>.

33. A peptide having the amino acid sequence:

(Peptide IXD)  
Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

wherein X is —OH or —NH<sub>2</sub>.

34. An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

(i) a solid phase coated with a peptide composition containing any one of the following peptides:

(Peptide I)  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X;

(Peptide IIF)  
Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

-continued

(Peptide II)

Ser—Gly—Lys—Pro—Ala—Ile—Ile—Pro—Asp—Arg—Glu—  
Val—Leu—Tyr—Arg—Glu—Phe—Asp—Glu—Met—Glu—  
Glu—Cys—Ser—Gln—His—Leu—Pro—Tyr—Ile—Glu—Gln—  
Gly—Met—Met—Leu—Ala—Glu—Gln—Phe—Lys—Gln—Lys—  
Ala—Leu—Gly—Leu—X;

(Peptide III)

Cys—Val—Val—Ile—Val—Gly—Arg—Val—Val—Leu—Ser—  
Gly—Lys—Pro—Ala—Ile—Ile—Pro—Asp—Arg—Glu—Val—  
Leu—Tyr—Arg—Glu—Phe—Asp—Glu—Met—Glu—Glu—  
Cys—Ser—Gln—His—Leu—Pro—Tyr—Ile—X;

(Peptide IIID)

Ser—Gly—Lys—Pro—Ala—Ile—Ile—Pro—Asp—Arg—Glu—  
Val—Leu—Tyr—Arg—Glu—Phe—Asp—Glu—Met—Glu—  
Glu—Cys—Ser—Gln—His—Leu—Pro—Tyr—Ile—X;

(Peptide IV)

Ser—Gly—Lys—Pro—Ala—Ile—Ile—Pro—Asp—Arg—Glu—  
Val—Leu—Tyr—Arg—Glu—Phe—Asp—Glu—Met—Glu—  
Glu—Cys—Ser—Gln—His—Leu—Pro—Tyr—Ile—Gln—  
Gly—Met—Met—Leu—Ala—Glu—Gln—Phe—X;

(Peptide V)

Lys—Gln—Lys—Ala—Leu—Gly—Leu—Leu—Gln—Thr—Ala—  
Ser—Arg—Gln—Ala—Glu—Val—Ile—Ala—Pro—Ala—Val—  
Gln—Thr—Asn—Trp—Gln—Lys—Leu—Glu—Thr—Phe—Trp—  
Ala—Lys—His—Met—Trp—Asn—Phe—X;

(Peptide VI)

Glu—Gln—Gly—Met—Met—Leu—Ala—Glu—Gln—Phe—  
Lys—Gln—Lys—Ala—Leu—Gly—Leu—Leu—Gln—Thr—Ala—  
Ser—Arg—Gln—Ala—Glu—Val—Ile—Ala—Pro—Ala—Val—  
Gln—Thr—Asn—Trp—Gln—Lys—Leu—Glu—Thr—X;

(Peptide VII)

Pro—Gly—Ala—Leu—Val—Val—Gly—Val—Val—Cys—Ala—  
Ala—Ile—Leu—Arg—Arg—His—Val—Gly—Pro—Gly—Glu—  
Gly—Ala—Val—Gln—Trp—Met—Asn—Arg—Leu—Ile—Ala—  
Phe—Ala—Ser—Arg—Gly—Asn—His—Val—Ser—Pro—X;

(Peptide VIII)

Ser—Thr—Ile—Pro—Lys—Pro—Gln—Arg—Lys—Thr—Lys—  
Arg—Asn—Thr—Asn—Arg—Arg—Pro—Gln—Asp—Val—Lys—  
Phe—Pro—Gly—Gly—Gly—Gln—Ile—Val—Gly—Gly—Val—  
Tyr—Leu—Leu—Pro—Arg—Arg—Gly—Pro—Arg—Leu—  
Gly—Val—Arg—Ala—Thr—Arg—Lys—Thr—Ser—Glu—Arg—  
Ser—Gln—Pro—Arg—Gly—Arg—Arg—X; and

(Peptide IXD)

Ile—Pro—Lys—Val—Arg—Arg—Pro—Glu—Gly—Arg—Thr—  
Trp—Ala—Gln—Pro—Gly—Tyr—Pro—Trp—Pro—Leu—Tyr—  
Gly—Asn—Glu—Gly—Cys—Gly—Trp—Ala—Gly—Trp—Leu—  
Leu—Ser—Pro—Arg—Gly—Ser—Arg—Pro—Ser—Trp—Gly—  
Pro—Thr—Asp—Pro—Arg—Arg—Arg—Ser—Arg—Asn—Leu—  
Gly—X

or mixtures thereof;

- (ii) a negative control sample; and
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.

35. An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

- (i) a solid phase coated with a peptide composition comprising a mixture of

(Peptide II)

Ser—Gly—Lys—Pro—Ala—Ile—Ile—Pro—Asp—Arg—  
Glu—Val—Leu—Tyr—Arg—Glu—Phe—Asp—Glu—Met—  
Glu—Glu—Cys—Ser—Gln—His—Leu—Pro—Tyr—Ile—  
Glu—Gln—Gly—Met—Met—Leu—Ala—Glu—Gln—Phe—  
Lys—Gln—Lys—Ala—Leu—Gly—Leu—X;

-continued

and

(Peptide V)

5 Lys—Gln—Lys—Ala—Leu—Gly—Leu—Leu—Gln—Thr—  
Ala—Ser—Arg—Gln—Ala—Glu—Val—Ile—Ala—Pro—  
Ala—Val—Gln—Thr—Asn—Trp—Gln—Lys—Leu—Glu—  
Thr—Phe—Trp—Ala—Lys—His—Met—Trp—Asn—Phe—X;

- (ii) a negative control sample; and
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.

36. An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

- (i) a solid phase coated with a peptide composition comprising a mixture of

(Peptide II)

20 Ser—Gly—Lys—Pro—Ala—Ile—Ile—Pro—Asp—Arg—  
Glu—Val—Leu—Tyr—Arg—Glu—Phe—Asp—Glu—Met—  
Glu—Glu—Cys—Ser—Gln—His—Leu—Pro—Tyr—Ile—  
Glu—Gln—Gly—Met—Met—Leu—Ala—Glu—Gln—Phe—  
Lys—Gln—Lys—Ala—Leu—Gly—Leu—X;

25 and

(Peptide VIII)

30 Ser—Thr—Ile—Pro—Lys—Pro—Gln—Arg—Lys—Thr—  
Lys—Arg—Asn—Thr—Asn—Arg—Arg—Pro—Gln—Asp—  
Val—Lys—Phe—Pro—Gly—Gly—Gly—Gln—Ile—Val—  
Gly—Gly—Val—Tyr—Leu—Leu—Pro—Arg—Arg—Gly—  
Pro—Arg—Leu—Gly—Val—Arg—Ala—Thr—Arg—Lys—  
Thr—Ser—Glu—Arg—Ser—Gln—Pro—Arg—Gly—Arg—  
Arg—X;

- (ii) a negative control sample; and
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.

37. An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

- (i) a solid phase coated with a peptide composition comprising a mixture of

(Peptide II)

45 Ser—Gly—Lys—Pro—Ala—Ile—Ile—Pro—Asp—Arg—Glu—  
Val—Leu—Tyr—Arg—Glu—Phe—Asp—Glu—Met—Glu—  
Glu—Cys—Ser—Gln—His—Leu—Pro—Tyr—Ile—Glu—Gln—  
Gly—Met—Met—Leu—Ala—Glu—Gln—Phe—Lys—Gln—Lys—  
Ala—Leu—Gly—Leu—X;

(Peptide V)

50 Lys—Gln—Lys—Ala—Leu—Gly—Leu—Leu—Gln—Thr—Ala—  
Ser—Arg—Gln—Ala—Glu—Val—Ile—Ala—Pro—Ala—Val—  
Gln—Thr—Asn—Trp—Gln—Lys—Leu—Glu—Thr—Phe—Trp—  
Ala—Lys—His—Met—Trp—Asn—Phe—X; and

(Peptide VIII);

60 Ser—Thr—Ile—Pro—Lys—Pro—Gln—Arg—Lys—Thr—Lys—  
Arg—Asn—Thr—Asn—Arg—Arg—Pro—Gln—Asp—Val—Lys—  
Phe—Pro—Gly—Gly—Gly—Gln—Ile—Val—Gly—Gly—Val—  
Tyr—Leu—Leu—Pro—Arg—Arg—Gly—Pro—Arg—Leu—  
Gly—Val—Arg—Ala—Thr—Arg—Lys—Thr—Ser—Glu—Arg—  
Ser—Gln—Pro—Arg—Gly—Arg—Arg—X;

- (ii) a negative control sample; and
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.

UNITED STATES PATENT AND TRADEMARK OFFICE  
CERTIFICATE OF CORRECTION

PATENT NO. : 5,106,726

Page 1 of 3

DATED : April 21, 1992

INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

At Column 4, lines 17 - 19: the first three lines of the amino acid sequence

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --.

At column 23, lines 46 - 47: the first two lines of the amino acid sequences:

"Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Tyr-  
Trp-Alg-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-  
Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --.



UNITED STATES PATENT AND TRADEMARK OFFICE  
CERTIFICATE OF CORRECTION

PATENT NO. : 5,106,726

Page 2 of 3

DATED : April 21, 1992

INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claim 1 (viii), the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --

In the Claim 30, the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 5,106,726

Page 3 of 3

DATED : April 21, 1992

INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --

Signed and Sealed this  
Twentieth Day of September, 1994

Attest:

*Bruce Lehman*

BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks

UNITED STATES PATENT AND TRADEMARK OFFICE  
CERTIFICATE OF CORRECTION

PATENT NO. : 5,106,726  
DATED : April 21, 1992  
INVENTOR(S) : Chang Yi Wang

Page 1 of 3

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

At Column 9, lines 17 - 19: the first three lines of the amino acid sequence

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- ---.

At column 23, lines 46 - 47: the first two lines of the amino acid sequences:

"Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Tyr-  
Trp-Alg-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-  
Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --

UNITED STATES PATENT AND TRADEMARK OFFICE  
CERTIFICATE OF CORRECTION

PATENT NO. : 5,106,726

Page 2 of 3

DATED : April 21, 1992

INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 49, lines 17-19:

In the Claim 1 (viii), the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-

Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --

Column 58, lines 18-20:

In the Claim 30, the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 5,106,726

Page 3 of 3

DATED : April 21, 1992

INVENTOR(S) : Chang Yi Wang


It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

should read: -- Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-  
Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --

This certificate Supersedes Certificate of Correction issued September 20, 1994.

Signed and Sealed this  
Thirteenth Day of December, 1994

Attest:



BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks

PATENT APPLICATION SERIAL NO. 07/558799

U.S. DEPARTMENT OF COMMERCE  
PATENT AND TRADEMARK OFFICE  
FEE RECORD SHEET

0620043 08/16/90 07558799 13-4500 020 101 155.00CH

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077558799



ABSTRACT

The present invention relates to a method for the detection in body fluids of antibodies to hepatitis C virus (HCV), also known as a non-A non-B hepatitis (NANBH) virus and to the diagnosis of NANBH by the use of a composition of synthetic peptides. Each of these peptides has an amino acid sequence corresponding to immunodominant regions of a fusion protein and a non-structural polypeptide of HCV, SOD/HCV C100 and a postulated HCV structural (core) protein. More specifically, the present invention is directed to the use of a group of synthetic peptides in a prescribed sequence or their analogues for the detection of antibodies to HCV in body fluids. The detection method includes an enzyme-linked immunosorbent assay (ELISA), and other forms of immunoassay procedures.

The present invention also relates to a method for generating high titer antibodies to HCV in healthy mammals, including humans, by the use of compositions containing these synthetic peptides, analogues or mixtures thereof, in a free, conjugated or polymeric form as key components in synthetic vaccines for the prevention of non-A non-B hepatitis (NANBH).

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UNITED STATES PATENT APPLICATION

Of

Chang Yi Wang

for

501

SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION  
OF ANTIBODIES TO HCV, DIAGNOSIS OF HCV INFECTION  
AND PREVENTION THEREOF AS VACCINES.

CROSS-REFERENCE TO RELATED APPLICATION

This is a continuation in part application of  
copending application Serial No. 07/481,348, <sup>and now abandoned</sup> filed February 16,  
1990, and application Serial No. 07/510,153, <sup>and now abandoned</sup> filed April 16,  
1990.

INTRODUCTION

The present invention relates to peptide compositions specific for the diagnosis and prevention of hepatitis C virus (HCV) infection, or non-A non-B hepatitis (NANBH). More particularly, the present invention is directed to synthetic peptide compositions which are specific for the detection of antibodies to HCV in body fluids and immunoassays using the same. The invention also includes the use of the synthetic peptide compositions as antigens for eliciting the production of monoclonal and polyclonal antibodies against HCV and as immunogens in vaccines for the prevention of NANBH or HCV infection.



1 In the 1940s, two independent investigators concluded  
2 that there were at least two types of viral hepatitis,  
3 designated as A and B (HAV and HBV) and that infection by one  
4 type, either HAV or HBV, did not confer the patient with  
5 cross-immunity (1-3). It was only in the 1970's with the  
6 introduction of serologic markers for hepatitis A and hepatitis  
7 B that it became possible to identify diseases caused by the  
8 two viruses and to distinguish between these two types of  
9 hepatitis clinically and serologically.

10 Subsequently, in 1974, Prince et al. suggested that  
11 many cases of transfusion hepatitis could not be attributed to  
12 HAV or HBV and were caused by an agent other than these  
13 viruses. They proposed naming the agent hepatitis C virus  
14 (HCV) (4). The presence of another hepatitis causing agent was  
15 subsequently confirmed by Alter et al., who reported that  
16 although the exclusion of commercial blood donors found to  
17 carry hepatitis B surface antigen (HBsAg) significantly reduced  
18 the frequency of post-transfusion hepatitis (5), 7 to 10  
19 percent of the 5 million Americans who received transfusions  
20 each year still developed hepatitis. In 90% of these  
21 post-transfusion hepatitis cases, a specific virus, unrelated  
22 to HAV, HBV, Epstein-Barr virus, cytomegalovirus or other  
23 viruses which occasionally produce liver diseases, was  
24 implicated as the etiologic agent (5). This infection was  
25 designated as non-A non-B hepatitis (NANBH).

26 Over the years, NANBH has been reported in patients  
27 undergoing hemodialysis, recipients of renal transplants (6),  
28 intravenous drug abusers (7) and patients in institutions for  
29 the mentally retarded (8). Further, nurses caring for patients  
30 with NANBH have also been found to contract this disease.

1 Epidemiologic evidence suggests that there may be  
2 three types of NANBH: the water-borne epidemic type; the blood  
3 or needle associated type; and the sporadically occurring  
4 community acquired type. However, the number and precise  
5 nature of the causative agents of NANBH still remain not  
6 entirely clear.

7 The acute phase of NANBH is less severe than that of  
8 hepatitis B, and the disease is rarely fatal. However, more  
9 than a third of the individuals who contract NANBH develop a  
10 chronic form of the disease in which they may remain infectious  
11 indefinitely. This chronic state may lead to cirrhosis of the  
12 liver and eventually to liver cancer.

13 Many methods have been developed in an attempt to  
14 detect the putative NANBH viral antigens and antibodies. These  
15 include agar-gel diffusion, counter immunoelectrophoresis,  
16 immunofluorescence microscopy, immunoelectron microscopy,  
17 radioimmunoassay, and enzyme-linked immunosorbent assay using  
18 crude biologic lysates and antibodies from patients. However,  
19 none of these assays are sufficiently sensitive, specific, and  
20 reproducible for use as a diagnostic test for NANBH. Some of  
21 the reactivities detected were later attributable to the  
22 presence of antibodies to host cytoplasmic antigens or low  
23 levels of a rheumatoid-factor-like substance frequently present  
24 in patients with or without hepatic diseases.

25 In the absence of a definitive test for NANBH, the  
26 diagnosis in the past has been one of exclusion. It was based  
27 on the clinical presence of acute hepatitis and the persistent  
28 absence of serologic markers for hepatitis A and B,  
29 Epstein-Barr virus or cytomegalovirus.

1           Because no specific test for the detection of  
2 antibodies to NANBH or HCV has been available, the use of  
3 nonspecific tests to screen donors has been adopted in the past  
4 decade as a means of preventing at least some post-transfusion  
5 NANBH.

6           One such surrogate test measures liver enzyme levels.  
7 The concentrations of some of the liver enzymes, in particular  
8 alanine aminotransferase (ALT), are frequently elevated in the  
9 blood of patients with active hepatitis. Two independent  
10 studies have shown a correlation between donor ALT levels and  
11 the incidence of NANBH in transfusion recipients (9-11).  
12 However, some studies showed that only about 20 percent of  
13 blood donors who transmitted NANBH have elevated liver enzyme  
14 concentrations. Other investigators, furthermore, have found  
15 that the liver enzyme levels can be increased by extraneous  
16 factors, such as heavy drinking.

17           Epidemiologic circumstances predisposing donor  
18 populations to infection with hepatitis B virus may also favor  
19 exposure to NANBH agents. A study conducted by Stevens et al.  
20 (12) evaluated the risk factors in donors for the presence of  
21 antibodies to hepatitis B virus. The results indicated that  
22 units of blood which were positive for antibodies to the  
23 hepatitis B core antigen (anti-HBc) appeared to present a two  
24 to three-fold greater risk of NANBH in the recipients than  
25 units without anti-HBc. They concluded that anti-HBc screening  
26 of donors might prevent about one third of the cases of NANBH  
27 attributable to transfusion, whereas ALT screening might  
28 prevent nearly one half of the cases of post transfusion NANBH.

29           Even with the use of these surrogate tests to  
30 establish the diagnosis of NANBH by exclusion, the correct

1 identification of the NANBHV carriers was still far from  
2 satisfactory. Firstly, there are a significant number of  
3 patients who received blood lacking the surrogate markers and  
4 yet developed NANBH. Secondly, there is a minimal overlap  
5 between donors with elevated ALT levels and those with  
6 anti-HBc. Lastly, there are recipients of blood units which  
7 were positive for a surrogate marker, but who did not become  
8 infected with NANBHV, also known as HCV (13-15).

9 Thus, there is an urgent demand for a sensitive and  
10 specific method to identify carriers of NANBHV and to screen  
11 out contaminated blood or blood products. In addition, there  
12 is also a need for an effective vaccine and/or therapeutic  
13 agent for the prevention and/or treatment of the disease.

14 Recently, a group of scientists at Chiron Corp.  
15 constructed a random-primed complementary DNA (cDNA) library  
16 from plasma containing the uncharacterized NANBH agent (16).  
17 They screened the library with serum from a patient diagnosed  
18 with NANBH and isolated a cDNA clone that encodes an antigen  
19 associated specifically with NANBH. This clone was found to be  
20 derived from the genome of an agent similar to the togaviridae  
21 or flaviviridae (16). The newly identified NANBH agent was  
22 called hepatitis C virus (HCV). A specific assay for this  
23 blood-borne NANBH virus was developed based on a fusion  
24 polypeptide of human superoxide dismutase (SOD) and 363 HCV  
25 amino acids, designated as SOD/HCV C100-3 (17). SOD/HVC ~~C-100~~<sup>C100-3</sup>  
26 was cultured from a clone of recombinant yeast, purified, and  
27 used to capture circulating viral antibodies (17). A family of  
28 cDNA sequences derived from this hepatitis C virus was  
29 subsequently reported in detail (18).  
30

1           However, the neucleotide sequence of HCV disclosed by  
2 the Chiron group covers only about 75% of the HCV genome and  
3 represents only the nonstructural genes.

4           More recently Mayumi, et al. determined the  
5 5'-terminal sequence of the genome of HCV for two distinct HCV  
6 strains in human and chimpanzee carriers (27). The 5'-terminal  
7 sequence contained a 5' non-coding region of at least 324  
8 nucleotides, well preserved in the two strains. The non-coding  
9 region was followed by a coding region of 1348 nucleotides  
10 continuing beyond the reported sequence of the prototype HCV  
11 which spanned 7310 nucleotides (18). Based on these results  
12 (18,27), HCV is considered to possess an uninterrupted open  
13 reading frame encoding at least 2886 amino acid residues.

14           A comparison of the complete nucleotide sequence of  
15 the Hepatitis C Virus to that of other Flaviviruses (28) has  
16 led us to postulate that two structural genes encoding for the  
17 core (or nucleocapsid protein) and the envelope proteins were  
18 contained in the HCV genome located in the upstream and  
19 downstream region respectively of the 5'-terminal sequence as  
20 reported by the Mayumi group (27). By careful analysis of the  
21 whole HCV genome structure and the predicted amino acid  
22 sequence encoded in the structural and non-structural proteins,  
23 we have now identified and characterized by an extensive series  
24 of experiments and through serological validation, the  
25 immunodominant regions of the HCV proteins.

26           The predicted amino acid sequence of the HCV genome is  
27 presented in Table 6, wherein the sequence for (a) is the  
28 sequence for J-1 (27, 29), (b) is the sequence for J-4 (27) and  
29 (c) is the sequence for the prototype PT (18). These show  
30

where conservative substitutions, deletions or substitutions  
can be made.

Table 6

(a)	MSTIPKPQRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRATR	50
(b)	---N-----	
	KTTERSQPRGRRQPIPKVRRPEGRTWAQPGYPWPLYGNEGCGWAGWLLSP	100
	-----W-----A-----A-----L-----	
	RGSRPSWGPTDPRRRSRNLGKVIDTLTCGFADLMGYIPLVGAPLGAARA	150
	-----	
	LAHGVRVLEDGVNYATGNLPGCSFSIFLLALLSCLTVPASAYQVRNSTGL	200
	-----I-----E---VS-I	
	YHVTNDPCNSSIVYEAHDAIHTPGCVPCVREGNVSRWVAMTPTVATRD	250
	-----S-----A-M-M-----D-S-----L---L-A-N	
	GKLPATQLRRHIDLLVGSATLCSALYVGDLCGSVFLIGQLFTFSPRRHWT	300
	ASV-T-TI---V-----A-AF---M-----VS-----E-	
	TQGCNCSIYPGHITGHRMAWDMMNWSPTAALVMAQLLRIPQAILDMIAG	350
	V-D-----LS-----T---VS-----VV--V--	
	AHWGVLAGIAYFSMVGNWAKVLVLLLFAGVDAETIVSGGQAARAMGLV	400
	-----L--Y-----I-A-----YT---A-SHTT-T-A	
	SLFTPGAQKNIQLINTNGSWHINSTALNCNESLNTGWLAGLIYQHKFNSS	450
	---S---S-R---V-----R-----D--H--F--A-F-T-R---	
(a)	GCPERLASCRRLTDFDQGWGPISHANGSGPDQRPYCWHYPPKPCGIVPAK	500
(b)	-----M-----IDW-A-----TYTEPDS-----A-R-----S	
(c)	-----Y-----	
	SVCGPVYCFTPSP	550
	Q-----	
	-----VVVGTTDRSGAPTYSWGENDTDFVLNNTRPPLGNWF	
	GCTWMNSTGFTKVCGAPPCVIGGAGNNTLHCPTDCFRKHPDATYSRCGSG	600
	PWITPRCLVDYPYRLWHWPCTINYTIFKIRMYVGGVEHRLEAACNWTRGE	650
	RCDLEDNRSELSPLLLTTTQWQVLPCSFTTLPALSTGLIHLHQNIVDVQ	700
	YLYGVGSSIASWAIKWEYVLLFLLLDARVCCLWMLLISQAEALQN	750
	LVILNAASLAGTHGLVSFLVFFCFAWYLGKGVPGAVYTFYGMWPLLLLL	800
	LALPQRAYALDTEVAASCGGVVLVGLMALTLSPYYKRYISWCLWWLQYFL	850
	TRVQAQLHVWIPPLNVRGGRDAVILLMLAVHPTLVFDITKLLAVFGPLW	900
	ILQASLLKVPWFVRVQGLLRFCALARKMIGGHYVQMVIKLGALTGTYYVY	950

1	NHLTPLRDWAHNGLRDLAVAVEPVVFSQMETKLITWGADTAACGDIINGL	1000
2	PVSARRGREILLGPADGMVSKGWRLAPITAYAQQTRGLLGCIITSLTGR	1050
3	DKNQVEGEVQIVSTAAQTFLATCINGVCWTVYHGAGTRTIA SPKGPVIQM	1100
4	YTNVDQDLVGWPAPQGSRLTPCTCGSSDLXLVTRHADVIPVRRRGASRG	1150
5	SLLSPROISYLGSSGGPLLCPAGHAVGIFRAAVCTRGVAKAVDFIPVEN	1200
6	LETTMRSPVFTDNSSPPVVPQSFQVAHLHAPTGSGKSTKVPAAYAAQGYK	1250
7	VLVLNPSVAATLFGAYMSKAHGIDPNIRTGVRTITTGSPITYSTYGKFL	1300
8	ADGGCSGGAYDIIICDELHSTDATSILGIGTVLDQAETAGARLVVLATAT	1350
9	PPGSVTVPHPNIEVALSTTGEIPFYGKAIPLEV IKGGRHLIFCHSKKKC	1400
10	(a) -	1450
11	(c) DELAAKLVALGINAVAYYRGLDVSVIPTSGDVVVVA'DALMTGYTGDFDS	
12	-----T-Y-----RR-----	1500
13	VIDCNTCVTQTVDFSLDPTFTIETITLPQDAVSRTQRRGRTGRGKPGIYR	
14	--T-----A-----S-----L-----	1550
15	FVAPGERPSGMFDSVLCYDAGCAWYELTPAETTVRLRAYMNTPLPV	
16	-----S-----A-D-F-----K--	1600
17	CNDHLEFWEGVFTGLTHIDAHFLSQTKQSGENLPYLVAQATVCARAQAP	
18	-----V-----I--	1650
19	PPSWDMWKCLIRLKPTLHGPTPLLYRLGAVQNEITLTHPVTKYIMTCMS	
20	ADLEVVTSTWVLVGGVLAALAAYCLSTGCVVIVGRVVLSGKPAIIPDREV	1700
21	LYREFDEMEECSQLPHYIENGMMLAENFKQKALGLLQTASRQAEVIAPAV	1750
22	QTNWQKLETFWAKHMWNFISGIQYLAGLSTLPGNPAIASLMAFTAAVTSP	1800
23	LTTSQTLLEFNILGGWVAAQLAAPGAATAFVGAGLAGAAIGSVGLGKVLID	1850
24	ILAGWGAGVAGALVAFKIMSGEVPSTEDLVNLLPAILSPGALVVGVCVAA	1900
25	ILRRHVGPGEAVNWMNRLIAFASRGNHVSPTHYVPESDAAARVAILSS	1950
26	LTVTQLLRRLHQWISSECTTPCSGSWLRDIWDWICEVLSDFKTWLKAKLM	2000
27	PQLPGIPFVSCQRGYKGVWRVDGIMHTRCHCGAEITGHVKNGTMRIVGPR	2050
28	TCRNMWSGTFPINAYTTGPCTPLPAPNYTFALWRVSAEYVEIRQVGDFH	2100
29	YVTGMTDNLKCPCQVPSPEFFTEL DGVRLHRFAPPCKPLLEEVSRVVG	2150
30	LHEYVGSQPLCEPEPDVAVLTSMLTDP SHITAEAGRRRLARGSPPSVAS	2200
	SSASQLSAPSLKATCTANHDSFDAELIEANLLWRQEMGGNITRVESENKV	2250
	VILDSFDPLVAEEDEREISVPAEILRKSRRFAQALPVWARPDYNPPLVET	2300

1	WKKPDYEPVHGCPLPPPKSPVPPPRKKRTVVLTESTLSTALAEATR	2350
2	SFGSSSTSGITGDNNTTSSEPAPSGCPPDSDAESYSSMPPLEGEPGDPDL	2400
3	SDGSWSTVSSEANAEDVVCCSMSYSWTGALVTPCAAEEQKLPINALSNSL	2450
4	LRHHNLVYSTTSRSACQRQKKVTFDRLQVLDSHYQDVLKEVKAAASKVKA	2500
5	NLLSVEEACSLTPPHSAKSKFGYGAKDVRCHARKAVTHINSVWKDLLEDN	2550
6	VTPIDTTIMAKNEVFCVQPEKGGRKPARLIVFDPDLGVRVCEKMALYDVVT	2600
7	KLPLAVMGSSYGFQYSPGQRFVFLVQAWKSKTTPMGFSYDTRCFDSTVTE	2650
8	SDIRTEEAIIYQCCDLDPQARVAIKSLTERLYVGGPLTNSRGENCYRRCR	2700
9	ASRASGVLTTSCGNLTLCYIKARAACRAAGLQDCTMLVCGDDLTVICESA	2750
10	GVQEDAASLRAFTEAMTRYSAAPPGDPPQPEYDLELITSCSSNVSAHDGA	2800
11	GKRVYYLTRDPTTPLARAAWETARHTPVNSWLGNII MFAPTLWARMILMY	2850
12	HFFSVLIARDQLEQALDCEIYGACYSIEPLDLPPIIQRL	2889

Synthetic peptides have been increasingly used to map antigenic or immunogenic sites on the surface of proteins, an approach recently termed "site-directed-serology". The present inventor (Wang, C.Y.) and a colleague have taken this approach to identify and characterize highly antigenic epitopes on the envelope proteins of HIV and to develop sensitive and specific immunoassays for the detection of antibodies to HIV (previously designated HTLV-III) (19-21). See also U.S. Patent 4,735,896, issued April 5, 1988 and U.S. Patent 4,879,212 issued Nov. 7, 1989, the contents of which are, hereby, fully incorporated by reference (22, 23). Subsequently, a series of finely mapped and well-characterized HTLV-I/II related synthetic peptides were employed in the development of synthetic peptide-based diagnostic assays for the detection of HTLV-I/II antibodies in infected individuals (24, 25). See also U.S. Patent 4,833,071 issued May 23, 1989, U.S.S.N. 07/297,635 filed January 13, 1989 and USSN 07/469,294 filed January 24, 1990. These assays have



1 provided superior sensitivity, excellent specificity, and, in  
2 certain cases, an unmatched capability to differentiate  
3 infections with two closely related viruses, thus overcoming  
4 many of the existing problems associated with biologically-  
5 derived tests based on either viral lysate or recombinant  
6 DNA-derived protein.

7 It is, therefore, an objective of the present  
8 invention to develop a detection or diagnostic procedure to  
9 identify and monitor HCV infection early in the disease cycle.

10 Another objective is to develop a test procedure that  
11 is highly sensitive and accurate.

12 A further objective is to chemically synthesize a test  
13 reagent which can then be used to detect the presence of  
14 antibodies to HCV in body fluids and diagnose NANBH.

15 Another objective is to develop a vaccine which, when  
16 introduced into healthy mammals, including humans, will  
17 stimulate production of efficacious antibodies to HCV, thereby  
18 providing protection against HCV infection.

19 A further objective is to provide a synthetic  
20 immunogen which can be used in mammals for the development of  
21 monoclonal and polyclonal antibodies to HCV.

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15  
16 BRIEF DESCRIPTION OF THE INVENTION  
17

18 According to the present invention, a series of  
19 synthetic peptides representing immunodominant regions of the  
20 hepatitis C virus (HCV) proteins, each arranged in a specific  
21 sequence, has been identified and made by solid phase peptide  
22 synthesis. These peptides have been found to be useful in a  
23 highly sensitive and accurate method for the early detection of  
24 antibodies to HCV in sera and body fluids and the diagnosis of  
25 non-A non-B hepatitis (NANBH). Because of their high  
26 immunoreactivity, it is expected that these peptides are also  
27 useful in stimulating production of antibodies to HCV in  
28 healthy mammals such as Balb/C mice, and in a vaccine  
29 composition to prevent HCV or NANBHV infection.  
30

According to the present invention, a peptide composition useful for the detection of antibodies to HCV and diagnosis of NANBH comprises a peptide selected from the group of peptides with the following sequences:

- a*
- (i) <sup>*Cys-Ser*</sup>  
Glu-Glu-~~Ser~~-Cys-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X (I)
- (ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (II)
- (iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)
- (iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X (III)
- (v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X (IV)
- 10X* (vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X (V)
- (vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X (VI)
- (viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X (VII)
- a* (ix) <sup>*Asp*</sup>  
Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-His-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X, and (VIII)

a 1 (x) ✓ Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-  
2 ✓ Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-  
3 Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-  
4 Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-  
5 Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-  
6 Gly-X (IX)

7 wherein X is -OH or -NH<sub>2</sub>, and analogues, segments, mixtures,  
8 combinations, conjugates and polymers thereof.

9 The amino acids in this application are abbreviated as  
10 shown herein below:

11 A= Ala= alanine,  
12 R= Arg= arginine,  
13 D= Asp= Aspartic acid,  
14 N= Asn= asparagine,  
15 Q= Gln= glutamine,  
16 E= Glu= glutamic acid,  
17 L= Leu= leucine,  
18 K= Lys= lysine,  
19 H= His= histidine,  
20 T= Thr= threonine,  
21 G= Gly= glycine,  
22 I= Ile= isoleucine,  
23 F= Phe= phenylalanine,  
24 S= Ser= serine,  
25 W= Trp= tryptophan,  
26 Y= Tyr= tyrosine,  
27 V= Val= valine,  
28 C= Cys= cysteine,  
29 P= Pro= proline  
30



1 An example of a combination is: Cys-Val-Val-Ile-Val-  
 2 Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-  
 3 Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-  
 4 Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-  
 5 Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-  
 6 Pro-X wherein X is -OH or -NH<sub>2</sub>. An example of a segment of  
 7 Peptide II is: Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-  
 8 Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-  
 9 Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X wherein X  
 10 is -OH or -NH<sub>2</sub> (IIF). An example of a segment of Peptide III  
 11 is:  
 12 Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-  
 13 Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X  
 14 wherein X is -OH or -NH<sub>2</sub> (IIID). An example of a segment of  
 15 Peptide IX is Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-  
 16 Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-  
 17 Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-  
 18 Gly-X (IXC).

19 The present invention also includes a highly sensitive  
 20 and accurate method of detecting antibodies to HCV in body  
 21 fluids and of diagnosing NANBH comprises the following steps:

22 A. Preparing a peptide composition comprising a  
 23 peptide selected from the group having the following amino acid  
 24 sequences:

- 25 (i) <sup>Cys-Val</sup> Glu-Glu-Ser-Cys-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-  
 26 Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-  
 27 Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-  
 Val-Ile-Ala-Pro-X (I)
- 28 (ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-  
 29 Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-  
 30 Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-  
 Gln-Lys-Ala-Leu-Gly-Leu-X (II)

- 1 (iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-  
2 Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-  
3 Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-  
4 Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)  
5  
6 (iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-  
7 Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-  
8 Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-  
9 Leu-Pro-Tyr-Ile-X (III)  
10  
11 (v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-  
12 Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-  
13 Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-  
14 Ala-Glu-Gln-Phe-X (IV)  
15  
16 (vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-  
17 Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-  
18 Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-  
19 Met-Trp-Asn-Phe-X (V)  
20  
21 (vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-  
22 Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-  
23 Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-  
24 Gln-Lys-Leu-Glu-Thr-X (VI)  
25  
26 (viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-  
27 Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-  
28 Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-  
29 Arg-Gly-Asn-His-Val-Ser-Pro-X (VII)  
30  
31 (ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-  
32 His-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-  
33 Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-  
34 Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-  
35 Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-  
36 Arg-X, and (VIII)  
37  
38 (x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-  
39 Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-  
40 Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-  
41 Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-  
42 Gly-Pro-Thr-Asp-Pro-Arg-Arg-Ser-Arg-Asn-Leu-  
43 Gly-X (IX)

wherein X is -OH or -NH<sub>2</sub>, and analogues, segments, mixtures, combinations, conjugates and polymers thereof; and

B. Using an effective amount of the peptide composition as the antigen in an immunoassay procedure.

Further, according to the present invention, the peptides by themselves, or when coupled to a protein or a polymeric carrier of homo or hetero dimers or higher oligomers

1 by use of homo or hetero functional multivalent cross linking  
2 reagents, or when directly synthesized and conjugated to a  
3 branching polyvalent lysine resin, can be used to elicit the  
4 production of antibodies to HCV in healthy mammals, including  
5 humans.

6 The method comprises introducing an effective amount  
7 of the peptide composition containing each of the individual  
8 peptides, analogues or segments or a mixture or a combination  
9 thereof, or in a polymeric form, into the body of a healthy  
10 mammal by intraperitoneal or subcutaneous injection.

11 Vaccines containing the peptides according to the  
12 present invention as the key immunogen may also be prepared.  
13 It is expected that such vaccine compositions may be useful to  
14 prevent HCV infection or NANBH.

15  
16 **BRIEF DESCRIPTION OF THE DRAWINGS**

17  
18 Figs. 1-1, 1-2, 1-3 and 1-4 show the amino acid  
19 sequences of the immunodominant region of a HCV non structural  
20 protein and precisely delineates the amino acid residues  
21 (underlined to show --- marginal, — moderate, and — strong)  
22 that contribute to the immunoreactivities of these HCV  
23 peptides with four representative HCV antibody positive sera.  
24 The immunoreactivities were measured as absorbance at 492nm by  
25 an EIA procedure.

26 Figs 2-1 and 2-2 are comparisons of the signal to  
27 cutoff ratio between the peptide based HCV-EIA employing only  
28 the non-structural protein sequence derived Peptide IIG of the  
29 present invention and that of the recombinant SOD/HCV C-100<sup>C/100-3</sup>  
30 protein based HCV-EIA. In Fig. 2-1 a well-characterized HCV

antibody positive control at various serum dilutions was used as the test sample. In Fig. 2-2 a panel of serum specimens derived from serial bleedings of a single individual spanning a period of sero-conversion to anti-HCV reactivity were used as samples.

Figs. 3-1 and 3-2 depict the frequency distribution of the HCV-EIA positivity, using Peptide IIG, represented by the signal to cutoff ratios obtained with 264 normal serum and 264 normal plasma specimens from commercial sources. The mean s/c ratios for the negative (n=250) and screened out positive (i.e. n=14) serum specimens are 0.034 and 7.202 respectively; and for the negative (n=255) and positive (n=9) normal plasma specimens the mean s/c ratios are 0.084 and 7.089 respectively.

Fig. 4 is a histogram depicting the immunoreactivities of Peptide IIG with sera from individuals: (a) positive for HBsAg, (n=50); (b) positive for antibodies to HBc protein, (n=39); (c) with elevated ( $>100$  I.U./L) alanine aminotransferase (ALT) enzyme activity, (n=174); (d) positive for antibodies to retroviruses HIV-1 (n=100), HIV-2 (n=10), HTLV-I/II (n=14); all asymptomatic, (total n=124); (e) with AIDS, ARC (N=200) or ATL (n=170) disease, (total n=270); and (f) with autoimmune disease (n=20).

Fig. 5 provides a comparison between EIA results using the Peptides IIF and IIID of the present invention and recombinant SOD/HCV <sup>C100-3</sup> ~~C-100~~, as represented by their respective s/c ratios on a panel of repeatably reactive specimens (n=23) obtained from a random donor population.

Figure 6 provides a comparison between a passive hemagglutination assay (PHA), using Peptide IIG, and the recombinant SOD/HCV <sup>C100-3</sup> ~~C-100~~, EIA as represented by their

6  
1        respective P/C and s/c ratios for a panel of SOD/HCV <sup>C/100-3</sup> ~~C-100~~ HCV  
2        EIA repeatably reactive specimens (n=20) obtained from a random  
3        donor population. For results obtained by the PHA, the  
4        agglutination pattern is quantitated by a specially designed  
5        optical reading instrument (manufactured by Olympus  
6        Corporation) where a P/C ratio of larger than 20 is considered  
7        negative whereas a P/C ratio of less than 20 is considered  
8        positive.

9        Figure 7-1 provides a study of serum samples collected  
10       over a ten year period of time from a NANBH patient who  
11       sero-converted after receiving HCV infected blood. The samples  
12       were tested by two EIA formats designated as A (coated with  
13       Peptides IIF and IIID at 5 ug/mL each) and B (coated with  
14       Peptides IIF, IIID and V at 5 ug/mL each) for comparison. The  
15       serum samples were provided by Dr. H. Alter of NIH.

16       Figure 7-2 provides a kinetic study with serum  
17       samples, kindly provided by Dr. C. Stevens of New York Blood  
18       Center, from a hemodialysis patient who sero-converted and  
19       contracted NANBH. These were tested by EIA format B (coated  
20       with peptides IIF, IIID and V at 5 ug/mL each).

21       Figure 7-3 provides a second kinetic study with serum  
22       samples, kindly provided by Dr. D. Bradley of Center for  
23       Disease Control, from a chimpanzee which sero-converted after  
24       being inoculated with a well-characterized strain of HCV and  
25       contracted NANBH, also tested by EIA format B.

26       Figures 8-1 and 8-2 depict the signal/cutoff ratio  
27       frequency distribution of both negative and positive serum  
28       specimens by a HCV-EIA format B. The results were obtained  
29       using 2035 low risk random blood donor specimens screen tested  
30       in a blood bank setting.

1           Figure 9 illustrates the inhibition by Peptide IV (an  
2 analogue) of binding of HCV specific antibodies to plates  
3 coated with peptides IID and IIIF at 5 ug/mL each at various  
4 peptide IV concentrations.

5           Figure 10 provides a comparison between the peptide  
6 based HCV EIA (coated with Peptide IIH and V at 10 and 5 ug/mL  
7 respectively) and recombinant protein based HCV EIA using  
8 samples from 74 hemodialysis patients, kindly provided by  
9 investigators at the Japanese National Institute of Health.

10          Figures 11-1, and 11-2 show the amino acid sequences  
11 of an immunodominant region of the postulated HCV structural  
12 (core or nucleocapsid) protein and precisely delineates the  
13 amino acid residues that contribute to the immunoreactivities  
14 of these HCV peptides with four representative HCV antibody  
15 positive sera (Samples 1-4). The immunoreactivities were  
16 measured as absorbance at 492nm by an EIA procedure.

17          Figure 12-1, 12-2 and 12-3 are histograms depicting  
18 the frequency distribution of HCV positivity in 221 sera from  
19 individuals: (a) with AIDS, ARC (n=63); (b) positive for  
20 HBsAg, (n=50); (c) positive for antibodies to HBe protein,  
21 (n=22); (d) with elevated ( $>100$  I.U./L) alanine  
22 aminotransferase (ALT) enzyme activity, (n=86) tested using  
23 three HCV EIA formats using Peptides IIH, V and VIIIE at 5, 3,  
24 and 2 ug/mL respectively (Format C); Peptides VIIIE, and IXD at  
25 2 and 2 ug/mL each (Format D), and Peptides IIH and V at 5 and  
26 3 ug/mL each (Format A).

27          Figures 13-1, 13-2, 13-3, 13-4, 13-5, and 13-6 depict  
28 the signal to cutoff ratio frequency distribution of HCV  
29 positivity in low risk random donor specimens using three  
30 HCV-EIA Formats, A (13-1 and 13-2), C (13-3 and 13-4), and D

1 (13-5 and 13-6). The results were screen tested in a blood  
2 bank setting.

3 Figure 14-1 provides a study of serum samples  
4 collected over a ten year period of time from a NANBH patient  
5 who sero-converted after receiving HCV infected blood. The  
6 samples were tested by a third EIA format designated as C  
7 (coated with Peptides IIH, V, and ~~VIII~~<sup>VIII E</sup> at 5, 3 and 2 ug/mL  
8 respectively) in comparison to two other EIA formats  
9 (designated as A and B.)

10 Figure 14-2 provides another kinetic study with serum  
11 samples, kindly provided by Dr. D. Bradley of Center for  
12 Diseases Control, from a chimpanzee which sero-converted after  
13 being inoculated with a well-characterized strain of HCV and  
14 contracted NANBH. These samples were tested by the HCV EIA  
15 Format C, in comparison to a RIA using rDNA based HCV C-100  
16 protein as the antigen. The ALT levels are also indicated with  
17 each bleed as a reference parameter.

18 Figures 15-1 and 15-2 both provide a side-by side data  
19 comparison via x-y plots with samples from hemodialysis  
20 patients, kindly provided by investigators at the Japanese  
21 National Institute of Health. The results were obtained by  
22 using the peptide based HCV EIA Format C (coated with peptides  
23 derived from both the structural and non-structural proteins  
24 containing IIH, V and ~~VIII~~<sup>VIII E</sup> at 5, 3, and 2 ug/mL respectively),  
25 HCV EIA Format A (coated with peptides derived from the  
26 nonstructural protein region containing IIH and V at 5 and  
27 3ug/mL respectively), and the recombinant HCV C-100 protein  
28 based EIA.

1 The amino acids in the drawings and tables are  
2 abbreviated using the art accepted single letter codes as  
3 follows:

4 A=Ala=alanine,  
5 R=Arg=arginine,  
6 D=Asp=aspartic acid,  
7 N=Asn=asparagine,  
8 Q=Gln=glutamine,  
9 E=Glu=glutamic acid,  
10 L=Leu=leucine,  
11 K=Lys=lysine,  
12 H=His=histidine,  
13 T=Thr=threonine,  
14 G=Gly=glycine,  
15 I=Ile=isoleucine,  
16 F=Phe=phenylalanine,  
17 S=Ser=serine,  
18 W=Trp=tryptophan,  
19 Y=Tyr=tyrosine,  
20 V=Val=valine,  
21 C=Cys=cysteine,  
22 P=Pro=proline,  
23 M=Met=methionine

24 DETAILED DESCRIPTION OF THE INVENTION

25  
26 In accordance with the present invention, three  
27 peptides and their segments have been chemically synthesized  
28 for the detection of antibodies to HCV in body fluids, the  
29 diagnosis of NANBH, and for the vaccination of healthy mammals  
30



by stimulating the production of antibodies to HCV. These peptides are arranged in the following sequences:

- a
- (i) <sup>Cys-Met</sup> Glu-Glu-Ser-Cys-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X (I)
- (ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (II)
- (iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)
- (iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X (III)
- (v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X (IV)
- (vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X (V)
- (vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X (VI)
- (viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X (VII)
- a
- (ix) <sup>Asn</sup> Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X; and (VIII)
- a
- (x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X (IX)

wherein X is -OH or -NH<sub>2</sub>.

1           These peptides may comprise combinations or segments,  
2 i.e. longer or shorter peptide chains by having more amino  
3 acids added to the terminal amino acids, or by amino acids  
4 removed from either terminal end.

5           These peptides may also comprise analogues to  
6 accommodate strain-to-strain variations among different  
7 isolates of HCV. HCV is indicated to have frequent mutations.  
8 Therefore, it is expected that variant strains, such as J-1 and  
9 J-4 (see reference #27), exist. Adjustments for conservative  
10 substitutions and selection among the alternatives where  
11 non-conservative substitutions are involved, may be made in the  
12 prescribed sequences. It is expected that as long as the  
13 peptide's immunoreactivity recognizable by the antibodies to  
14 HCV is preserved, analogues of the synthetic peptide may also  
15 comprise substitutions, insertions and/or deletions of the  
16 recited amino acids of the above sequence.

17           These peptides may also comprise conjugates, i.e.,  
18 they may be coupled to carrier proteins such as bovine serum  
19 albumin (BSA) or human serum albumin (HSA). Furthermore, these  
20 peptides may comprise polymers, i.e., they may be synthesized  
21 on a polymeric resin, such as a branching octameric lysine  
22 resin.

23           The amino acid sequences of the polypeptides useful as  
24 test reagents for the detection of antibodies to HCV in body  
25 fluids and diagnosis of NANBH are selected to correspond to a  
26 partial segment of the amino acid sequence of the HCV  
27 proteins: a non-structural protein designated as HCV  
28 C-100(18), and a structural protein such as the core  
29 (nucleocapsid) protein (27).  
30

In selecting regions of the HCV protein for epitope analysis, peptides in the 40mer size range with amino acid sequences covering the complete HCV C-100 protein and the postulated core protein were synthesized. These were tested for their immunoreactivity with serum from a patient positively diagnosed with HCV infection. Six overlapping peptides from the HCV C-100 protein region designated as I, II, III, IV, V and VI and two adjacent peptides from the postulated core protein region designated as VIII and IX were identified to have specific immunoreactivity with the positive HCV serum. Another peptide VII and its fragments, C-terminal to this immunodominant region, was also found to have moderate immunoreactivity with a sub population of HCV positive sera. See Example 12. Peptide IIH, another analogue of Peptide II, with five additional amino acids to the N-terminus has been found to be highly immunogenic and contains an additional epitope recognizable by antibodies in sera from patients with acute phase NANBHV infection (with elevated ALT levels). The amino acid sequences of the peptides are as follows:

- a*
- (i) Glu-Glu-<sup>Cys-Val</sup>~~Ser-Cys~~-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X (I)
- 10X* (ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (II)
- (iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)
- (iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X (III)

- 1 (v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-  
2 Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-  
3 Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-  
4 Ala-Glu-Gln-Phe-X (IV)  
5 (vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-  
6 Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-  
7 Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-  
8 Met-Trp-Asn-Phe-X (V)  
9 (vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-  
10 Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-  
11 Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-  
12 Gln-Lys-Leu-Glu-Thr-X (VI)  
13 (viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-  
14 Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-  
15 Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-  
16 Arg-Gly-Asn-His-Val-Ser-Pro-X (VII)  
17 (ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-  
18 His-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-  
19 Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-  
20 Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-  
21 Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-  
22 Arg-X, and (VIII)  
23 (x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-  
24 Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-  
25 Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-  
26 Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-  
27 Gly-Pro-Thr-Asp-Pro-Arg-Arg-Ser-Arg-Asn-Leu-  
28 Gly-X (IX)

29 The six peptides I, II, III, IV, V and VI span a  
30 region of 90 amino acids:

31 Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-  
32 Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-  
33 Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-  
34 Gln-Pro-Lys-Lys-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-  
35 Arg-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-  
36 Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe

37 and were found to have specific immunoreactivity with the  
38 positive control serum. Table 1 shows the amino acid sequence  
39 of this immunodominant region of the HCV protein, and presents  
40 the amino acid sequence of the six chemically synthesized  
41 peptides, designated as I to VI and segments (A to H) thereof.

1 Another two peptides (VIII and IX) spanning a region  
2 of 119 amino acids located inside the 5' terminal of the  
3 postulated HCV core protein:

4 Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-<sup>Asn</sup>Thr-Asn-Arg-  
5 Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-  
6 Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Alg-Thr-  
7 Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-  
8 Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Alg-Gln-Pro-Gly-Tyr-  
9 Pro-Trp-Pro-Leu-<sup>Asn</sup>Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Alg-Gly-Trp-Leu-  
10 Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-  
11 Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

12 were found to have specific immunoreactivity with a  
13 representative panel of well-characterized HCV antibody  
14 positive sera.

15 Table 7 shows the amino acid sequence of this  
16 immunodominant region of the postulated HCV core protein, and  
17 presents the amino acid sequence of the ten chemically  
18 synthesized peptides. They were designated, as Peptides VIII  
19 and IX with segments (A to D) thereof. Each of these peptides  
20 was coated at 5ug/mL in a 10mM sodium bicarbonate buffer (pH  
21 9.5) onto polystyrene microwell plates and tested in a three  
22 step 45 minute enzyme immunoassay procedure, described  
23 hereinbelow, with a panel of HCV antibody  
24 positive sera, each selected as representative  
25 of a particular clinical population, at  
26 various serum dilutions.

Table 1

CHARACTERIZATION OF THE IMMUNODOMINANT REGION OF THE HCV SOD-C100 FUSION POLYPEPTIDE:

	CV, VTIVGR, VVLSG, KPATII, POREV, LYREF, DEMEE, CSQHL, PYIEQ, GMILA, EQFKQ, KALGL, LOTAS, RQAEV, IAPAV, QTNNQ, KLETF, WAKHM, WNF	RELATIVE (%) IMMUNOREACTIVITY
IA	GL, LOTAS, RQAEV, IAP	3.0
IB	KQ, KALGL, LOTAS, RQAEV, IAP	10.3
IC	MLA, EQFKQ, KALGL, LOTAS, RQAEV, IAP	23.9
ID	EQ, GMILA, EQFKQ, KALGL, LOTAS, RQAEV, IAP	24.6
IE	HL, PYIEQ, GMILA, EQFKQ, KALGL, LOTAS, RQAEV, IAP	38.2
IF	EE, CSQHL, PYIEQ, GMILA, EQFKQ, KALGL, LOTAS, RQAEV, IAP	45.6
IJA	GMILA, EQFKQ, KALGL	3.1
IJB	PYIEQ, GMILA, EQFKQ, KALGL	24.3
IJC	CSQHL, PYIEQ, GMILA, EQFKQ, KALGL	41.7
IJD	DEMEE, CSQHL, PYIEQ, GMILA, EQFKQ, KALGL	44.9
IJE	LYREF, DEMEE, CSQHL, PYIEQ, GMILA, EQFKQ, KALGL	57
IJF	POREV, LYREF, DEMEE, CSQHL, PYIEQ, GMILA, EQFKQ, KALGL	99
IIG	II, POREV, LYREF, DEMEE, CSQHL, PYIEQ, GMILA, EQFKQ, KALGL	93.2
IIH	SG, KPATII, POREV, LYREF, DEMEE, CSQHL, PYIEQ, GMILA, EQFKQ, KALGL	101
IIIA	EF, DEMEE, CSQHL, PYI	4.9
IIIB	EV, LYREF, DEMEE, CSQHL, PYI	26.3
IIIC	II, POREV, LYREF, DEMEE, CSQHL, PYI	85
IIID	SG, KPATII, POREV, LYREF, DEMEE, CSQHL, PYI	100
IIIE	GR, VVLSG, KPATII, POREV, LYREF, DEMEE, CSQHL, PYI	99
IIIF	CV, VTIVGR, VVLSG, KPATII, POREV, LYREF, DEMEE, CSQHL, PYI	95
IV	SG, KPATII, POREV, LYREF, DEMEE, CSQHL, PYIEQ, GMILA, EQFKQ	93.8
VIA	AS, RQAEV, IAPAV, QTNNQ, KLETF	3.9
VIB	GL, LOTAS, RQAEV, IAPAV, QTNNQ, KLETF	43.6
VIC	KQ, KALGL, LOTAS, RQAEV, IAPAV, QTNNQ, KLETF	44.0
VID	LA, EQFKQ, KALGL, LOTAS, RQAEV, IAPAV, QTNNQ, KLETF	46.0
VIE	EQ, GMILA, EQFKQ, KALGL, LOTAS, RQAEV, IAPAV, QTNNQ, KLETF	54.8
VA	AV, QTNNQ, KLETF, WAKHM, WNF	1.3
VB	EV, IAPAV, IAPAV, QTNNQ, KLETF, WAKHM, WNF	17.8
VC	AS, RQAEV, IAPAV, QTNNQ, KLETF, WAKHM, WNF	23.4
VD	GL, LOTAS, RQAEV, IAPAV, QTNNQ, KLETF, WAKHM, WNF	32.3
VE	KQ, KALGL, LOTAS, RQAEV, IAPAV, QTNNQ, KLETF, WAKHM, WNF	93.9

The underlined amino acid residues exert ( — ) marginal, ( — ) moderate, or ( — ) strong immunoreactivity

Table 7

Identification and characterization of An Immunodominant Region in HCV's Structural Proteins, based on the predicted amino acid sequence derived from the structural genes of two HCV genomes (27), by serological validation with a combination of synthetic peptides (VIII-E and IXA-E):

J-1 J-4	Peptide Code	Peptide Sequence	% Relat Immuno -react
J-1	VIII E	STIP, KPQRK, TKRNT, NRRPQ, DVKEP, GGGQI, VGGVY, LLPRR, GPRLG, VRATR, KTSEK, SQRPG, RR	98.6
	VIII D	TKRNT, NRRPQ, DVKEP, GGGQI, VGGVY, LLPRR, GPRLG, VRATR, KTSEK, SQRPG, RR	84.1
	VIII C	DVKEP, GGGQI, VGGVY, LLPRR, GPRLG, VRATR, KTSEK, SQRPG, RR	70.2
	VIII B	VGGVY, LLPRR, GPRLG, VRATR, KTSEK, SQRPG, RR	54.8
	VIII A	GPRLG, VRATR, KTSEK, SQRPG, RR	27.5
	IX E	G, RRPQI, PKVRR, PEGRT, HAQPG, YPMPL, YGNEG, CGHAG, WLLSP, RGSRP, SMGPT, DPRRR, SRNLG	49.5
	IX D	I, PKVRR, PEGRT, HAQPG, YPMPL, YGNEG, CGHAG, WLLSP, RGSRP, SMGPT, DPRRR, SRNLG	58.1
	IX C	I, HAQPG, YPMPL, YGNEG, CGHAG, WLLSP, RGSRP, SMGPT, DPRRR, SRNLG	57.1
	IX B	L, YGNEG, CGHAG, WLLSP, RGSRP, SMGPT, DPRRR, SRNLG	48.4
	IX A	WLLSP, RGSRP, SMGPT, DPRRR, SRNLG	23.8
J-4	VIII E	STIP, KPQRK, TKRNT, NRRPQ, DVKEP, GGGQI, VGGVY, LLPRR, GPRLG, VRATR, KTSEK, SQRPG, RR	98.6
	VIII D	TKRNT, NRRPQ, DVKEP, GGGQI, VGGVY, LLPRR, GPRLG, VRATR, KTSEK, SQRPG, RR	84.1
	VIII C	DVKEP, GGGQI, VGGVY, LLPRR, GPRLG, VRATR, KTSEK, SQRPG, RR	70.2
	VIII B	VGGVY, LLPRR, GPRLG, VRATR, KTSEK, SQRPG, RR	54.8
	VIII A	GPRLG, VRATR, KTSEK, SQRPG, RR	27.5
	IX E	G, RRPQI, PKVRR, PEGRT, HAQPG, YPMPL, YGNEG, CGHAG, WLLSP, RGSRP, SMGPT, DPRRR, SRNLG	49.5
	IX D	I, PKVRR, PEGRT, HAQPG, YPMPL, YGNEG, CGHAG, WLLSP, RGSRP, SMGPT, DPRRR, SRNLG	58.1
	IX C	I, HAQPG, YPMPL, YGNEG, CGHAG, WLLSP, RGSRP, SMGPT, DPRRR, SRNLG	57.1
	IX B	L, YGNEG, CGHAG, WLLSP, RGSRP, SMGPT, DPRRR, SRNLG	48.4
	IX A	WLLSP, RGSRP, SMGPT, DPRRR, SRNLG	23.8

~~procedure, described hereinbelow, with a panel of HCV antibody~~  
~~positive sera, each selected as representative of a particular~~  
~~clinical population, at various serum dilutions.~~ Calculations  
based on the overall EIA absorbance of all positive sera  
yielded an array of immunoreactivity indices represented as  
% relative immunoreactivity for each of the synthetic HCV  
peptides. Three peptides, designated as IIF, IIH and IIID,  
being 40 mer, 47 mer and 30 mer in size, with the following  
amino acid sequence respectively:

Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-  
Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-  
Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-  
Ala-Leu-Gly-Leu (IIF)

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-  
Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-  
Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-  
Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu (IIH)

and

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-  
Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-  
Gln-His-Leu-Pro-Tyr-Ile (IIID)

were found to have the highest immunoreactivity with the sera  
panel. The relative (%) immunoreactivity for each of the 40  
HCV peptides listed in Tables 1 and 7, as a result of this  
extensive epitope mapping study, provides a basis for the  
delineation of several clusters of amino acid residues (as  
underlined), each in a prescribed sequence, that are involved  
in or relevant to the antigenic configuration of the HCV  
peptides. Two peptides, designated as VIIIE and IXD being  
61mer and 56mer in size are respectively located within the HCV  
structural core protein region with the following amino acid  
sequences:



a 1 Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-~~His~~<sup>Asn-</sup>  
2 Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-  
3 Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-  
4 Gly-Pro-Arg-Leu-Gly-Val-Arg-Alg-Thr-Arg-Lys-Thr-Ser-  
5 Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X (VIII E)

a 6 ~~Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-~~  
7 ~~Gly-Arg-Thr-Trp-Alg-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-~~  
8 ~~Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Alg-Gly-Trp-Leu-Leu-~~  
9 ~~Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-~~  
10 ~~Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X~~ (IX D)

7 Peptides VIII E and IX D were also found to have the highest  
8 reactivity in this region.

9 Assays for antibodies to HCV based upon chemically  
10 synthesized peptides show several advantages over assays  
11 utilizing biologic based immunoabsorbents. The peptides can  
12 easily be synthesized in gram quantities by using automated  
13 solid-phase methods, thus providing a reproducible antigen of  
14 high integrity with consistent yields. The presence of other  
15 antigens from biological systems precludes such  
16 reproducibility. More importantly, non-specific reactivities  
17 seen in uninfected individuals are likely to be due to the  
18 heterogeneity of the preparations used for assay. This is  
19 particularly true for assays using biologically based  
20 immunoabsorbents. In these processes, the host antigens are  
21 frequently co-purified with the desired viral protein(s).  
22 Antibodies to these contaminating antigens are frequently found  
23 in normal individuals, thus resulting in false-positive results.

24 The assay of the present invention clearly minimizes  
25 such false-positive reactions as encountered in the other assay  
26 systems and, at the same time, shows a high sensitivity to  
27 truly positive sera by the substantially increased  
28 signal-to-noise ratio. This increased signal-to-noise ratio  
29 probably resulted from the purity of the immunoabsorbent. The  
30

1 assay of the present invention is also highly specific, in that  
2 the mean S/C ratios for HCV carriers are about 80-200 times the  
3 mean S/C of those of the non-infected individuals. For a  
4 representative example, see Figs. 3-1 and 3-2.

5 The peptides useful as solid phase immunoabsorbents  
6 for the detection of antibodies to HCV were synthesized by the  
7 "classical" Merrifield method of solid phase peptide synthesis  
8 using side chain protected t-Boc-amino acids to correspond to  
9 the following amino acid sequences:

- a
- 10 (i) Glu-Glu-<sup>Cys-Leu</sup>~~Ser-Cys~~-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-  
11 Gly-Met-~~Met~~-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-  
12 Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-  
Val-Ile-Ala-Pro-X (I)
- 13 (ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-  
14 Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-  
Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-  
Gln-Lys-Ala-Leu-Gly-Leu-X (II)
- 15 (iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-  
16 Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-  
17 Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-  
Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)
- 18 (iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-  
19 Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-  
Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-  
Leu-Pro-Tyr-Ile-X (III)
- 20 (v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-  
21 Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-  
22 Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-  
Ala-Glu-Gln-Phe-X (IV)
- 23 (vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-  
24 Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-  
Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-  
Met-Trp-Asn-Phe-X (V)
- 25 (vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-  
26 Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-  
27 Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-  
Gln-Lys-Leu-Glu-Thr-X (VI)
- 28 (viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-  
29 Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-  
Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-  
30 Arg-Gly-Asn-His-Val-Ser-Pro-X (VII)

a 1 (ix) <sup>asn</sup> Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-  
2 His-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-  
3 Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-  
Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-  
Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-  
Arg-X, and (VIII)

a 4 (x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-  
5 Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-  
6 Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-  
7 Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Ara-Pro-Ser-Trp-  
Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-  
Gly-X (IX)

1 8 wherein X is -NH<sub>2</sub>.

9 Other analogues, segments and combinations of these  
10 peptides may be prepared by varying the amino acid sequences  
11 either by adding, subtracting, substituting, or deleting  
12 desired t-Boc-amino acid(s).

13 Following completion of assembly of the desired  
14 blocked peptide on the resin, the peptide-resin is treated with  
15 anhydrous hydrofluoric acid to cleave the peptide from the  
16 resin. Functional groups of amino acids which are blocked  
17 during synthesis by benzyl-derived blocking groups are also  
18 cleaved from the peptide simultaneously. The free peptide is  
19 then analyzed and purified by high performance liquid  
20 chromatography (HPLC) and characterized biochemically by amino  
21 acid analysis.

22 Longer peptides with more than about 50 amino acids  
23 may also be prepared conveniently using well known recombinant  
24 methods. The known nucleic acids codons for each of the amino  
25 acids in the peptide may be utilized and synthetic genes  
26 encoding such peptides constructed. The synthetic gene may be  
27 inserted into vector constructs by known techniques, cloned and  
28 transfected into host cells, such as E. coli, or yeast. The  
29 secreted polypeptide may then be processed and purified  
30 according to known procedures. The peptides synthesized

1 according to the above described procedures are highly reactive  
2 with antibodies to HCV and can be used as a highly sensitive  
3 and specific immunoadsorbent for the detection of antibodies to  
4 HCV.

5 Figs. 1-1, 1-2, 1-3 and 1-4 and Figs 11-1 and 11-2  
6 show the amino acid sequences of the immunodominant regions of  
7 HCV proteins, both structural and non-structural, and precisely  
8 delineates, in the case of the non-structural protein HCV C-100  
9 region, the underlined amino acid residues that contribute (---  
10 marginally, — moderately, or —> strongly) to the  
11 immunoreactivities, measured at A492nm by a peptide based EIA  
12 procedure of these HCV peptides with four representative HCV  
13 antibody positive sera.

14 The peptide based EIA procedure used to measure the  
15 immunoreactivity of each peptide is as follows. 100uL per well  
16 of each of the peptides was coated at 5ug/mL in a pH 9.5 sodium  
17 bicarbonate buffer (10mM) onto a polystyrene microwell plate  
18 and the microwell plate was incubated at 37°C for about an  
19 hour, washed and dried. The test serum samples were diluted  
20 with PBS containing normal goat serum, gelatin and TWEEN 20.  
21 200uL of the test serum sample solution was added to each well  
22 and allowed to react for 15 mins. at 37°C. The wells were  
23 washed, enzyme labelled antibodies were used to bind the  
24 HCV-antibody-peptide complex, and the plate was incubated for  
25 another 15 min. A color developer, e.g. orthophenylenediamine  
26 (OPD), was then added. The reaction was stopped after 15 min  
27 by the addition of 50uL 1.0M H<sub>2</sub>SO<sub>4</sub>, and the absorbance of  
28 the reaction mixture was read at 492nm with an ELISA reader.

29 As demonstrated in Fig. 1-1, serum sample 1 has little  
30 reactivity with Peptide IA and IB. However, its reactivity

1 with Peptide IC increases significantly, followed by a marginal  
 2 increase with Peptide ID, and additional increases with  
 3 Peptides IE and IF. This indicates that in the HCV Peptide I  
 4 series, two clusters of amino acid residues, namely <sup>Leu-Ala-Glu-Gln-Phe and</sup> LAEQE and  
 5 <sup>His-Leu-Pro-Ser-Ile and</sup> HLPYI are contributing to the antigenic determinant(s) of the  
 6 HCV Peptide I. Similarly, a cluster of residues namely  
 7 <sup>His-Glu-Asp-Glu-Gln-His-Leu-Pro-Ser-Ile and</sup> EECSQHLPYI is contributing to the immunoreactivity of the HCV  
 8 Peptide II series; another cluster of residues namely  
 9 <sup>His-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg and</sup> SGKPAIIPDR is contributing to the immunoreactivity of HCV  
 10 Peptide III series and two clusters of residues, namely <sup>Gly-Leu-Leu-Gln-Ile and</sup> GLLQT and  
 11 <sup>His-Val-Ile-Ala-Pro and</sup> EVIAP are contributing to the immunoreactivity by HCV  
 12 peptides IV and V series. As shown on the bottom of Fig. 1-1,  
 13 a total of six spaced clusters of amino acid residues  
 14 representing discontinuous epitopes in this immunodominant  
 15 region of the HCV protein are identified as contributing to the  
 16 specific HCV immunoreactivity with serum sample 1.

17 Figure 1-2 illustrates an immunoreactivity profile for  
 18 serum sample 2 when tested on a total of 31 overlapping  
 19 peptides in the HCV Peptide I, II, III, IV, V and VI series.  
 20 There is a clear difference between the immunoreactivity  
 21 profiles of serum samples 1 and 2. The immunodominant epitope,  
 22 as marked by residues <sup>His-Gly-Lys-Pro-Ala and</sup> SGKPA and <sup>Ile-Ile-Pro-Pro-Asp-Arg-Glu-Val and</sup> IIPDREVA is located towards the  
 23 N-terminus of the region.

24 Figure 1-3 illustrates an immunoreactivity profile for  
 25 serum 3 when tested on the same 31 HCV peptide panel. Through  
 26 this extensive epitope mapping analysis, serum sample 3 was  
 27 found to have a similar immunoreactivity profile to that of  
 28 serum sample 2.

1           Figure 1-4 illustrates an immunoreactivity profile for  
2 serum sample 4 which differs significantly from that of sample  
3 2 and 3, while maintaining some similarity to that of sample 1.

4           In summary, epitope mapping analysis conducted with a  
5 series of 31 overlapping peptides covering an immunodominant  
6 region of the HCV non-structural protein, which spans a total  
7 of 90 amino acid residues as illustrated in Table 1, and an  
8 immunodominant region of the HCV structural core protein, which  
9 spans a total of 119 amino acid residues as illustrated in  
10 Table 7, reveals a varying degree of immunoreactivity among  
11 different HCV antibody positive samples and these HCV  
12 peptides. Based on overall EIA absorbance readings obtained  
13 with a panel of eight HCV positive sera with each of these 31  
14 HCV peptides (Table 2), a relative (%) immunoreactivity index  
15 is established for each of the peptides and several clusters of  
16 amino acid residues are identified as contributing strongly, as  
17 in the cases of Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg and  
18 Glu-Val-Ile-Ala-Pro; moderately, as in the cases of  
19 Ser-Gly-Lys-Pro-Ala, Glu-Val-Leu-Tyr-Arg-Glu-Phe,  
20 Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly; and  
21 Leu-Ala-Glu-Gln-Phe-Lys-Gln; or marginally, as in the case of  
22 Lys-Gln-Lys-Ala-Leu, to the HCV immunoreactivity.

23           Similarly, the relative immunoreactivity of Peptide  
24 VIII and IX and their analogue-segments are presented in Table  
25 7.

Table 2

HCV Peptide Analogues<sup>a</sup>

Specimens	I						II						III					
	A	B	C	D	E	F	A	B	C	D	E	F	A	B	C	D	E	F
Blank	0.041	0.041	0.041	0.041	0.041	0.041	0.041	0.040	0.040	0.041	0.041	0.041	0.040	0.046	0.040	0.045	0.040	0.043
NRC	0.047	0.050	0.049	0.049	0.052	0.055	0.045	0.044	0.048	0.046	0.048	0.088	0.044	0.048	0.061	0.073	0.071	0.070
WRC	0.040	0.048	0.077	0.084	0.155	0.221	0.040	0.042	0.220	0.153	0.241	0.399	0.046	0.043	0.192	0.243	0.269	0.222
SRC	0.049	0.055	0.330	0.383	0.828	1.175	0.043	0.093	1.188	0.963	1.279	1.832	0.046	0.074	1.081	1.260	1.379	1.127
1	0.066	0.218	1.925	2.151	2.994	3.247	0.075	0.838	3.219	3.282	3.494	3.316	0.289	0.527	3.245	4.057	3.545	3.613
2	0.054	0.095	0.080	0.093	0.171	0.337	0.066	0.103	0.243	0.536	0.872	2.929	0.191	0.316	2.715	2.941	3.053	2.984
3	0.062	0.099	0.062	0.064	0.068	0.108	0.065	0.058	0.121	0.129	0.371	2.406	0.066	0.085	2.407	2.612	2.566	2.624
4	0.082	1.068	1.391	1.912	1.994	2.726	0.074	2.769	2.387	2.437	2.822	3.289	0.064	2.864	3.096	3.221	3.319	3.220
5	0.063	0.083	0.136	0.156	0.246	0.216	0.057	0.065	0.104	0.085	0.197	0.732	0.056	0.126	0.588	0.657	0.700	0.522
6	0.059	0.073	0.058	0.066	0.071	0.071	0.061	0.068	0.066	0.061	0.086	0.623	0.054	0.075	0.458	0.623	0.641	0.489
7	0.050	0.052	0.058	0.062	0.091	0.091	0.046	0.049	0.066	0.048	0.152	1.146	0.045	0.273	0.863	1.577	1.669	1.505
8	0.070	0.087	0.254	0.293	0.710	0.698	0.070	0.076	0.718	0.812	1.463	1.998	0.058	0.101	0.655	0.894	0.937	0.820
8 A492nm	0.056	1.765	3.964	4.077	6.345	7.494	0.514	4.026	6.924	7.390	9.457	16.44	0.823	4.367	14.03	16.58	16.43	15.78
i=1																		
% Relative Immu- no- reactivity	3.0	10.3	23.9	24.6	38.2	45.6	3.1	24.3	41.7	42.9	57	95	93.2	4.9	26.3	85	100	99
																		95

1 Based on the above-mentioned epitope mapping study,  
2 four representative EIAs were configured using Peptide IIG  
3 alone, a mixture of two Peptides IIF and IIID, a mixture of  
4 IIF, IIID and V, or a mixture of IIH and V as the solid phase  
5 antigen.

6 Figs. 2-1 and 2-2 depict the comparison, by signal to  
7 cutoff ratio, between the peptide based HCV-EIA employing  
8 Peptide IIG, at 5 ug/mL coating concentration, and that of  
9 recombinant SOD/HCV C-100<sup>C/100-3</sup> protein based HCV-EIA. In Fig. 2-1,  
10 a well-characterized HCV antibody positive control at various  
11 serum dilutions was used as the sample. In Fig 2-2, a panel of  
12 serum specimens derived from serial bleedings of a single  
13 individual spanning a period of sero-conversion to anti-HCV  
14 reactivity was used. Similar dilution titers and equal ability  
15 to identify date of sero-conversion, the two parameters  
16 indicative of the sensitivity of each assay, are obtained with  
17 the synthetic peptide based EIA according to the present  
18 invention and rDNA HCV C-100 based EIA, except that the peptide  
19 based assay according to the present invention is more  
20 sensitive, conferring a higher signal to cutoff ratio to its  
21 positive specimens.

22 Fig. 3-1 and 3-2 depict the frequency distribution of  
23 the synthetic peptide based HCV-EIA signal to cutoff ratios,  
24 using Peptide IIG at 5ug/mL as the coating concentration,  
25 obtained with 264 normal serum and 264 normal plasma specimens  
26 from commercial sources. The mean s/c ratios for the negative  
27 (n=250) and screened out positive (i.e. n=14) serum specimens  
28 are 0.034 and 7.202 respectively; for the negative (n=255) and  
29 positive (n=9) normal plasma specimens the mean ratios are  
30 0.084 and 7.089 respectively. A sharp contrast between the



1 screened out positives and all the negatives is obtained with  
2 the peptide based HCV-EIA of the present invention.

3       Based on the high degree of sensitivity and  
4 specificity of the peptide compositions according to the  
5 present invention in their immunoreactivities to antibodies to  
6 HCV, it is believed that the peptide compositions according to  
7 the present invention may also be useful as vaccines to prevent  
8 NANBH, and as immunogens for the development of both monoclonal  
9 and polyclonal antibodies to HCV in mammals, including humans.  
10 The peptide compositions when coupled to a protein, or  
11 synthesized on a polymeric carrier resin (e.g., an octameric  
12 branching lysine resin) or when polymerized to homo or hetero  
13 dimers or higher oligomers by cysteine oxidation, induced  
14 disulfide cross linking, or by use of homo or hetero functional  
15 multivalent cross linking reagents, can be introduced to normal  
16 subjects to stimulate production of antibodies to HCV in  
17 healthy mammals.

18       The advantages of using the peptides according to the  
19 present invention are many.

20       Since the peptide compositions according to the  
21 present invention are not derived biologically from the virus,  
22 there is no danger of exposing the normal subjects who are to  
23 be vaccinated to the disease.

24       The peptides can be chemically synthesized easily.  
25 This means that there is no involvement with the HCV at any  
26 time during the process of making the test reagent or the  
27 vaccine. Another problem which can be minimized by the process  
28 of the present invention is the false positive results caused  
29 by the presence of antigenic materials from host cells  
30 co-purified with the HCV fusion protein. Certain normal

1 individuals have antibodies to E. Coli or yeast proteins which  
2 are cross reactive with the antigenic materials from host  
3 cells. Sera from these normal individuals may show a positive  
4 response in the immunoassays.

5 Further, with appropriate amino acid modifications or  
6 substitutions, it is expected that various peptide analogues  
7 based on the prescribed amino acid sequence can be synthesized  
8 with properties giving rise to lower background readings or  
9 better binding capacity to solid phases useful for HCV antibody  
10 screening assays.

11 Moreover, because the peptide compositions of the  
12 present invention are synthetically prepared, the quality can  
13 be controlled and as a result, reproducibility of the test  
14 results can be assured. Also, since very small amounts of  
15 peptides are required for each test procedure, and because the  
16 expense of preparing the peptides is relatively low, the cost  
17 of screening body fluids for antibodies to HCV, diagnosis of  
18 NANBH infection, or the preparation of a vaccine is relatively  
19 low.

20 The peptides prepared in accordance with the present  
21 invention can be used to detect HCV infection and diagnose  
22 NANBH by using them as the test reagent in an enzyme-linked  
23 immunoadsorbent assay (ELISA), an enzyme immunodot assay, an  
24 agglutination based assay, or other well-known immunoassay  
25 devices. The preferred method is ELISA. The ELISA technique  
26 is exemplified in Examples 1, 2, 8-10, 12 and 14-18 and the  
27 agglutination based assay in Examples 3 and 4. The Examples  
28 are used to illustrate the present invention and are not to be  
29 used to limit the scope of the invention.

1 It is to be noted that in the following methods, 0.25%  
2 by weight of glutaraldehyde may be added to the coating buffer  
3 to facilitate better peptide binding onto the plates or beads.  
4 Further, horseradish peroxidase (HRPO) conjugated mouse  
5 monoclonal anti-human IgG antibody or the HRPO conjugated  
6 second antibodies from any other animal species may be used in  
7 place of the HRPO-conjugated goat anti-human IgG as the second  
8 antibody tracer.

9 The gelatin used in these processes can include calf  
10 skin gelatin, pig skin gelatin, fish gelatin or any known  
11 available gelatin proteins, or be replaced with albumin  
12 proteins.

#### 13 EXAMPLE 1

14 Measurement of Relative (%) Immunoreactivity for  
15 synthetic peptide covering an immunodominant region of  
16 the HCV protein C-100 by an Enzyme-Linked Immunosorbent Assay

17 Wells or 96-well plates were coated at 4°C overnight  
18 (or 1 hour at 37°C), with each of the thirty one peptides: IA  
19 to IF, IIA to IIH, IIIA to IIIF, IV, V, VIA to VIE (see Table  
20 1) prepared as described at 5 ug/mL at 100 uL per well in 10mM  
21 NaHCO<sub>3</sub> buffer, pH 9.5. The peptide coated wells were then  
22 incubated with 250 uL of 3% by weight of gelatin in PBS at 37°C  
23 for 1 hour to block non-specific protein binding sites,  
24 followed by three washes with PBS containing 0.05% by volume of  
25 TWEEN 20 and then dried. The test specimens were diluted with  
26 PBS containing 20% by volume normal goat serum, 1% by weight  
27 gelatin and 0.05% by volume TWEEN 20 at dilutions of 1:20  
28 volume to volume, respectively. 200 uL of the diluted  
29 specimens were added to each of the wells and allowed to react  
30 for 15 minutes at 37°.

1 The wells were then washed six times with 0.05% by  
2 volume TWEEN 20 in PBS in order to remove unbound antibodies.  
3 Horseradish peroxidase conjugated goat anti-human IgG was used  
4 as a second antibody tracer to bind with the HCV  
5 antibody-peptide antigen complex formed in positive wells. 100  
6 uL of peroxidase labeled goat anti-human IgG at a dilution of  
7 1:1800 in 1% by volume normal goat serum, 0.05% by volume TWEEN  
8 20 in PBS was added to each well and incubated at 37°C for  
9 another 15 minutes.

10 The wells were washed six times with 0.05% by volume  
11 TWEEN 20 in PBS to remove unbound antibody and reacted with  
12 100uL of the substrate mixture containing 0.04% by weight  
13 orthophenylenediamine (OPD) and 0.12% by volume hydrogen  
14 peroxide in sodium citrate buffer, pH 5.0.

15 This substrate mixture was used to detect the  
16 peroxidase label by forming a colored product. Reactions were  
H 17 stopped by the addition of 100 uL of 1.0M H<sub>2</sub>SO<sub>4</sub> and the  
18 absorbance measured using an ELISA reader at 492nm (i.e.  
H 19 A<sub>492</sub>). Assays were performed in singlet at one specimen  
20 dilution (1:20) with a panel of eight representative HCV  
21 antibody positive sera, along with the specimen diluent blank,  
22 non-reactive, weakly reactive and strongly reactive controls  
23 (NRC, WRC, SRC) all in duplicates.

24 Results obtained from this study are shown in Table  
25 2. According to the EIA absorbance readings at 492nm (y axis)  
26 and the amino acid sequences for each of the corresponding HCV  
27 peptides (x axis), representative immunoreactivity profiles are  
28 plotted for four of the eight sera as shown in Figures 1-1 to  
29 1-4. Relative (%) immunoreactivity index for each of the 31  
30 peptides is calculated using Peptide IIID as a reference based

1 on the total absorbance of eight sera at 492nm (See Tables 1  
2 and 2). Fig. 1 shows the amino acid sequences of the  
3 immunodominant region according to data presented in Tables 1  
4 and 2, and precisely delineates the amino acid residues  
(underlined) that contribute (--- marginally, \_\_\_ moderately,  
5 and \_\_\_ strongly) to the immunoreactivities.

6  
7 In summary, epitope mapping analysis conducted with a  
8 series of 31 overlapping peptides covering an immunodominant  
9 region of HCV, spanning a total of 90 amino acid residues as  
10 illustrated in Table 1, reveals a varying degree of  
11 immunoreactivities between different HCV antibody positive  
12 samples and these HCV peptides. Based on this study, several  
13 discontinuous epitopes are located within this immunodominant  
14 region. Contrary to what is speculated by the conventional  
15 wisdom, it is found preferably to have peptides with longer  
16 amino acid chains, ideally longer than 20, synthesized in order  
17 to optimally present these antigenic determinants to HCV  
18 antibodies.

19 Based on the above-mentioned epitope mapping study,  
20 four representative EIAs using peptide IIG alone, or a mixture  
21 of Peptides IIF and IID, or a mixture of IIF, IIID and V, or a  
22 mixture of IIH and V as the solid phase antigen were  
23 configured for the following efficacy studies as demonstrated  
24 in Examples 2, 8, 9, 10 and 12.

#### 25 EXAMPLE 2

#### 26 Detection of Antibodies to HCV by an 27 Enzyme-Linked Immunosorbent Assay

28 Wells of 96-well plates were coated at 4°C overnight  
29 (or for 1 hour at 37°C) with either Peptide IIG alone at a  
30 coating concentration of 0.5ug per well (designated as IIG EIA)

1 or with a mixture of two Peptides IIF and IIID (designated as  
2 IIF/IIID EIA) in a ratio by weight of IIF:IIID=1:1 at 1 ug per  
3 well of the mixture in 100 uL 10mM NaHCO<sub>3</sub> buffer pH 9.5. The  
4 peptide coated wells were then incubated with 250 uL of 3% by  
5 weight of gelatin in PBS at 37°C for 1 hour to block  
6 non-specific protein binding sites, followed by three more  
7 washes with PBS containing 0.05% by volume of TWEEN 20 and  
8 dried.

9 The test specimens were diluted with PBS containing  
10 20% by volume normal goat serum, 1% by weight gelatin and 0.05%  
11 by volume TWEEN 20 at dilutions of 1:20 volume to volume,  
12 respectively. 200 uL of the diluted specimens were added to  
13 each of the wells and allowed to react for 15 minutes at 37°.

14 The wells were then washed six times with 0.05% by  
15 volume TWEEN 20 in PBS in order to remove unbound antibodies.  
16 Horseradish peroxidase conjugated goat anti-human IgG was used  
17 as a second antibody tracer to bind with the HCV  
18 antibody-peptide antigen complex formed in positive wells. 100  
19 uL of peroxidase labeled goat anti-human IgG at a dilution of  
20 1:1800 in 1% by volume normal goat serum, 0.05% by volume TWEEN  
21 20 in PBS was added to each well and incubated at 37°C for  
22 another 15 minutes.

23 The wells were washed six times with 0.05% by volume  
24 TWEEN 20 in PBS to remove unbound antibody and reacted with 100  
25 uL of the substrate mixture containing 0.04% by weight  
26 orthophenylenediamine (OPD) and 0.12% by volume hydrogen  
27 peroxide in sodium citrate buffer, pH 5.0. This substrate  
28 mixture was used to detect the peroxidase label by forming a  
29 colored product. Reactions were stopped by the addition of 100  
30 uL of 1.0M H<sub>2</sub>SO<sub>4</sub> and the absorbance measured using an ELISA

1 reader at 492nm (i.e.  $A_{492}$ ). Assays were performed in  
2 singlet at one specimen dilution (1:20) with all test  
3 specimens. Each plate run is accompanied by a panel of eight  
4 controls including the specimen diluent blank, negative, weak  
5 HCV reactive and strong HCV reactive controls, all in  
6 duplicate. The strongly reactive control was adjusted by  
7 diluting a HCV positive serum in the specimen dilution buffer  
8 at 1:300, which gave an absorbance value at 492nm of about 1.5  
9 when performed in this standard 45 minute assay procedure. A  
10 cutoff value is calculated based on the following formula:  
11  $\text{Cutoff} = (0.1 \times \text{SRC}) + \text{NRC}$ . Both the raw absorbance  
12 (designated as signal) and the ratio of signal to cutoff are  
13 recorded for all specimens analyzed.

14 The following groups of specimens were analyzed on the  
15 HCV peptide based EIA according to the present invention, with  
16 the plates coated either with 5 ug/mL of peptide IIG or a  
17 mixture containing 5ug/mL IIF and 5ug/mL IIB:

- 18 (a) A well-characterized HCV antibody positive control  
19 based on serum dilutions; (on both IIG and IIF/IIID  
20 EIAs)
- 21 (b) a panel of serum specimens derived from serial  
22 bleedings of a single individual spanning a period of  
23 sero-conversion to anti-HCV reactivity; (on both IIG  
24 and IIF/IIID plates)
- 25 (c) 264 normal serum and 264 normal plasma specimens from  
26 commercial sources; (on IIG plates only)
- 27 (d) individuals positive for HBsAg, (n=30); (on both IIG  
28 and IIF/IIID plates)
- 29 (e) individuals positive for antibodies to HBC protein,  
30 (n=39); (on both IIG and IIF/IIID plates)

- a 1 (f) individuals with elevated (<sup>></sup>100 I.U./L) alanine  
2 aminotransferase (ALT) enzyme activity, (n=174); (on  
3 both IIG and IIF/IIID plates)  
4 (g) individuals positive for antibodies to retroviruses  
5 HIV-1(n=100), HIV-2(n=10), HTLV-I/II(n=14); all  
6 asymptomatic, (total n=124); (on both IIG and IIF/IIID  
7 plates)  
8 (h) individuals with AIDS, ARC(n=200) or ATL (n=170)  
a 9 disease, (total <sup>n=370</sup>~~n=270~~); (on both IIG and IIF/IIID  
10 plates) and  
11 (i) individuals with autoimmune disease (n=20). (on IIG  
12 plates only)  
B 13 (j) recombinant SOD/HCV <sup>C100-3</sup>~~C-100~~ HCV-EIA repeatably reactive  
14 specimens obtained from a random donor population,  
15 (n=23). (on both IIG and IIF/IIID plates).  
16

17 Results obtained from groups (a) and (b) are presented  
18 in Figs. 2-1 and 2-2 respectively (data obtained on IIG plates  
19 only), from group (c) in Figs. 3-1 and 3-2; from groups (d) to  
20 (i) in Fig. 4, from group (j) in Table 3 and Figs. 5 and 6.

21 In brief, as shown in Figs. 2-1 and 2-2, a comparison,  
22 by signal to cutoff ratio, between the peptide based HCV-EIA of  
23 the present invention employing peptide IIG and that of  
B 24 recombinant SOD/HCV <sup>C100-3</sup>~~C-100~~ protein based HCV-EIA produced by  
25 Chiron/Ortho. Similar dilution titers and equal ability to  
26 identify date of sero-conversion, the two parameters indicative  
27 of each assay's sensitivity, are obtained for both assays.  
28 However, the assay according to the present invention is more  
29 sensitive and confers a higher signal to cutoff ratio to its  
30 positive specimens.



Table 3

SAMPLE ID No.	rDNA			ALT (IU/L)	Anti-HBc (S/C)	OTHER POSITIVES	Peptide HCV-EIA S/C
	HCV S/C	RPT S/C	RPT S/C				
1 161	5.33	5.56	5.56	36/56	2.10		11
2 280	5.76	5.56	5.56	78/56	0.07+	HBc, ALT	10
3 374	1.98	2.45	2.45	20/56	1.97		0.573
4 517	5.79	5.68	5.68	34/56	2.04		11
5 561	1.74	2.75	2.47	21/56	2.46		0.172
6 675	0.93	1.33	1.54	29/56	1.98		0.135
7 720	5.68	5.68	5.68	57/56	0.08+	HBc, ALT	13
8 773	5.56	5.88	5.88	86/56	2.07	HIV, ALT	8.625
9 797	3.79	4.35	4.29	74/56	0.38+	HBc, ALT	1.802
10 869	5.66	5.59	5.59	35/56	2.45		9.755
11 873	5.66	5.59	5.59	26/56	2.34		1.189
12 1003	1.63	1.24	1.01	31/56	2.02		0.078
13 1073	5.73	5.59	5.59	17/56	0.12+	HBc	2.594
14 1099	1.72	1.76	1.94	10/56	1.84		0.083
15 1118	5.59	5.79	5.79	10/56	0.31+	HBc	10.5
16 1336	0.93	1.38	1.38	18/56	2.15		0.010
17 1501	5.75	5.67	5.67	36/56	1.09		5.349
18 1530	1.27	1.48	1.50	23/56	2.30		0.943
19 1557	0.91	1.29	1.28	20/56	2.20		0.385
20 1652	2.06	2.64	2.72	42/56	1.72		0.135
21 1877	5.59	5.63	5.63	65/56	2.16	ALT	4.943
22 1940	1.64	1.47	1.17	29/56	2.35		0.052
23 2017	5.60	5.84	5.84	11/56	0.19+	HBc	6.786

Col. 1,2,3,=Ortho's HCV results in s/c.; Col 5=ALT values over cutoff in IU/L; Col. 6=Abbott's Anti-HBc results in s/c where results UNDER 1.00 are POSITIVE due the competitive binding principle of this assay.

1 As shown in Figs. 3-1 and 3-2, the frequency  
2 distribution of the HCV-EIA signal to cutoff ratios, using  
3 peptide IIG at 5ug/mL as the coating concentration, that was  
4 obtained with 264 normal serum and 264 normal plasma specimens  
5 for commercial sources suggested a repeatably reactive rate of  
6 5.3% and 3.4% respectively. These percentages are relatively  
7 high compared with those reported in field clinical trials  
8 (usually 0.5-1.0%) using the rDNA HCV C-100 based EIA kit  
9 (Chiron/Ortho). However, in the assay according to the present  
10 invention, the mean s/c ratios for the negative (n=250) and  
11 screened out positive (i.e. n=14) serum specimens are 0.034 and  
12 7.202 respectively; for the negative (n=255) and positive (n=9)  
13 normal plasma specimens the mean ratios are 0.084 and 7.089  
14 respectively. Such a sharp contrast between the screened out  
15 positives and all the negatives probably precludes the  
16 likelihood of a high false positive rate. Since these normal  
17 specimens are derived from commercial plasma centers where the  
18 paid donors usually represent a population with higher  
19 incidence of viral markers than the rigorously monitored blood  
20 banks, a higher repeatably reactive rate is also considered  
21 reasonable. Previous clinical studies indicated that between 7  
22 to 10 percent of patients receiving transfusions developed  
23 NANBH, where 90% of these post-transfusion hepatitis cases are  
24 caused by the NANBHV(5). These reports also provide some  
25 support to the interpretation of the data obtained herein that  
26 a high reactivity represents a true positive result.

27 Results obtained from the screening of a total of 677  
28 well-characterized clinical specimens previously categorized  
29 into six groups, from (d) to (i) using a representative lot of  
30

plates coated with Peptide IIG, were plotted on a histogram as shown in Fig. 4.

Fifteen out of fifty (i.e. 30%) HBsAg carriers, 3 out of 39 (i.e. 8%) HBe antibody positive individuals, 43 out of 174 (i.e. 24.7%) individuals with elevated ALT enzyme activity, 8 out of 124 (6.5%) asymptomatic individuals with retroviral antibodies, 6 out of 270 (i.e. 2.2%) individuals with retroviral related disease, and 0 out of 20 (i.e. 0%) individuals with autoimmune disease were found to be repeatably reactive with the peptide HCV EIA of the present invention using peptide IIG. All these positive specimens were also found to be positive when tested on peptides IIF/IIID HCV EIA, although with much higher s/c ratios.

A much higher percentage of positive cases was found with those who have abnormal liver functions (24.7%) or previous infection(s) with Hepatitis B (30% and 8%) when compared to those with other infections or diseases (e.g. 6.5%, 2.2% and 0%).

Note: Sera from HBsAg carriers were kindly provided by the Infectious Diseases Laboratory of the American Red Cross; sera from HBe antibody positive donors were obtained from Boston Biomedica Inc.; sera from individuals with elevated ALT levels ( $>100$  I.U./L) were obtained from both Boston Biomedica Inc. and NABI laboratory; sera from asymptomatic individuals with retroviral antibodies (HIV-1 and HTLV-1) were obtained from New York Blood Center, and those with HIV-2 antibodies were from Guinea Bissau of West Africa, kindly provided by Dr. O. Varnier of Italy; sera from patients with ATL were kindly provided by the Japanese

1 Red Cross; sera from patients with AIDS and ARC, were  
2 kindly provided by Dr. D. Knowles at Columbia  
3 University College of Physicians and Surgeons, and Dr.  
4 F. Siegal at the Long Island Jewish Hospital; sera  
5 from patients with various complications of autoimmune  
6 diseases were kindly provided by Dr. N. Chiorazzi of  
7 the Cornell University Medical School. All sera have  
8 been characterized by additional licensed serologic  
9 markers before inclusion in the current study.

10 Table 3 illustrates results obtained with the peptide  
11 based HCV EIA described in this invention on a panel of 23  
12 recombinant HCV EIA repeatably reactive specimens obtained from  
13 a random donor population. Data on each specimen's ALT level  
14 and HBc antibody reactivity are provided as supplemental  
15 information for indirect confirmation of NANBH status of the  
16 positive donors. As can be seen from the Table, all eight  
17 specimens with indirect confirmation of their NANBH status  
18 scored positive in the peptide based EIA according to the  
19 present invention (on both IIG and IIF/IIID plates). In  
20 addition, four specimens that scored high on the peptide based  
21 assay also scored as strong positives by the recombinant HCV  
22 EIA, thus further confirming the HCV positivity of these  
23 specimens. Only one specimen scored marginally positive on the  
24 peptide based HCV EIA, which lacks the other markers. However,  
25 this specimen scored positively with the recombinant HCV EIA.  
26 The remaining ten specimens that scored negative by the peptide  
27 based EIA according to the present invention all had a marginal  
28 s/cutoff ratio of between 0.9 to 2.6. Fig. 5 provides a direct  
29 correlation between the peptide based HCV EIA of the present  
30 invention and the recombinant based HCV EIA by their respective

1 s/cutoff ratios for this panel. Thus, the peptide based HCV  
2 EIA of the present invention can clearly differentiate the  
3 repeatably reactive specimens previously screened out by the  
4 rDNA based HCV EIA into two distinct groups, a positive group  
5 which correlated highly to those with other known NANBH markers  
6 and a negative group which probably represents specimens with  
7 extraneous reactivities unrelated to HCV. In addition to its  
8 use as a screening assay, the peptide based HCV EIA may also  
9 function as a positive confirmatory test for the rDNA based HCV  
10 EIA.

11  
12 Note: This well-characterized serum panel was kindly  
13 provided by Dr. C. Fang of the American Red Cross QC  
14 laboratory.

### 15 EXAMPLE 3

#### 16 Detection of Antibodies to HCV 17 By an Agglutination Based Assay

18 The presently claimed HCV peptides, synthesized  
19 according to the Merrifield solid phase method, can be  
20 conjugated to bovine serum albumin (BSA) by a simple  
21 crosslinking method in the presence of a low percentage of  
22 glutaraldehyde solution (0.025%), or with other crosslinking  
23 reagents such as m-maleimidobenzoyl-N-hydroxysuccinimide ester  
24 (MBS) according to a previously published procedure  
25 (Biochemistry, 18:690-697, 1979). For example: to 0.32 mL. of  
26 a BSA solution (10 mg/mL in 0.01 M phosphate buffer, pH 7.0) at  
27 room temperature is added 0.013 mL of an MBS solution (0.025  
28 mg/mL in dimethylformamide). The amount of MBS added to the  
29 BSA solution can be varied dependent on the optimal molar ratio  
30

1 of BSA to MBS determined for a specific conjugate studied. The  
2 mixture is stirred at room temperature for 1 hour, after which  
3 it is centrifuged to remove any precipitated albumin. The  
4 clarified mixture is then subjected to gel filtration on  
5 Sephadex G-25 and the protein-containing fractions, as detected  
6 by their absorbance at 280 nm, are pooled and stored frozen at  
7 -70°C until needed.

8 The peptides are dissolved in H<sub>2</sub>O at 10 mg/mL. A  
9 predetermined amount of each peptide solution is added dropwise  
10 to the previously activated BSA-MBS solution and stirred at  
11 room temperature for 3 hours. The final peptide-BSA conjugates  
12 are separated from other free peptides by gel filtration or  
13 extensive dialysis. The ratio of peptide to BSA is determined  
14 by SDS-PAGE according to conventional methods.

15 Using the above mentioned peptide-BSA conjugation  
16 process, conjugated peptide IIG-BSA was absorbed to double  
17 aldehyde fixed human O erythrocytes at pH 4.0. The  
18 peptide-conjugate coated erythrocytes were then treated with  
19 NaBH<sub>4</sub> to prevent non-specific protein binding. The  
20 peptide-conjugate coated erythrocytes were then washed with PBS  
21 and incubated with 5% normal human serum-PBS solution. These  
22 processed cells were then used in an agglutination assay for  
23 the detection of HCV antibodies in both serum and plasma  
24 specimens. The specimens were diluted 1:10 in a sample diluent  
25 buffer and an equal volume of the indicator cells (50 uL) was  
26 mixed with the diluted specimens. The agglutination pattern  
27 was settled within one hour; and the assay results were read by  
28 the naked eye and further quantitated by an optical device  
29 (manufactured by Olympus Corporation) which gave a P/C ratio,  
30 as determined by the absorbance readings of the periphery and

1 center of the wells. In this experiment, a P/C ratio of 20 was  
2 set as the assay cutoff value, i.e. a positive agglutination  
3 pattern had a ratio of  $\leq 20$  and a negative pattern,  $>20$ .

4 A total of 20 rDNA HCV EIA repeatably reactive  
5 specimens were tested for antibodies to HCV in the  
6 above-described HCV passive hemagglutination assay (PHA)  
7 employing Peptide IIG-BSA conjugate as the solid phase. Figure  
8 6 provides a correlation study between the peptide based HCV  
9 PHA and the recombinant based HCV EIA by their respective P/C  
10 and s/c ratios. All samples with s/c EIA ratios higher than 3  
11 were found to be positive with the HCV PHA test. With the  
12 exception of one, all specimens having borderline s/c ratios  
13 (between 0.9 to 2) scored as negative in this PHA test.

#### 14 EXAMPLE 4

15 Detection of Antibodies to HCV By An  
16 Agglutination Assay Utilizing As the Solid Phase  
17 Immunosorbent Gelatin Particles, Erythrocytes  
18 Of Different Animal Species, Or Latex Particles  
19 Coated with a Mixture of HCV Peptides

20 One mL thoroughly washed erythrocytes, gelatin  
21 particles, or polystyrene latex particles are coated with the  
22 HCV peptide mixture, or conjugates thereof at an effective  
23 concentration. The peptide mixture, or conjugates thereof,  
24 coated cells or particles are then incubated with serially  
25 diluted serum samples in the wells of a 96-well U-shaped  
26 microplate or on a slide. After being left at room temperature  
27 for about an hour, or a few minutes in the case of latex  
28 particle based microagglutination, the settled agglutination  
29 pattern on the bottom of each well or on the slide is read; and  
30 the highest dilution showing a positive reaction is recorded.

1 This is a one-step assay which can be used for both  
2 qualitative and quantitative detection of antibodies to HCV in  
3 specimens including sera or biofluids.

4  
5 EXAMPLE 5

6 A test kit for detecting HCV antibodies using an  
7 agglutination assay comprises a compartmented enclosure  
8 containing multiple microwell plates and other accessory  
9 materials for an agglutination assay including (1) a bottle of  
10 HCV peptide coated erythrocytes, gelatin particles or latex  
11 polystyrene particles; (2) a negative control; and, (3) an  
12 inactivated HCV positive control, and (4) specimen diluent.  
13 The procedure described in Examples 3 and 4 is to be followed.

14  
15 EXAMPLE 6

16 An enzyme immunoassay based diagnostic test kit for  
17 the detection of HCV antibodies can be constructed. The test  
18 kit comprises a compartmented enclosure containing multiple  
19 96-well plates coated prior to use with the HCV peptide or  
20 peptide mixtures of the present invention in 100 uL pH 9.5 10mM  
21  $\text{NaHCO}_3$  buffer. The kit further comprises materials for  
22 enzyme detection in separate sealed containers consisting of:  
23 1) a negative control; 2) an inactivated HCV positive control;  
24 3) specimen diluent; 4) peroxidase labeled-second antibody to  
25 human IgG; and 5) a color change indicator consisting of, for  
26 example, orthophenylenediamine (OPD) and hydrogen peroxide in a  
27 phosphate citrate buffer. The procedure described in Examples  
28 1 and 2 is to be followed.

29 In this test kit, 96-well plates, precoated with a  
30 peptide or peptide mixture of the present invention, can be



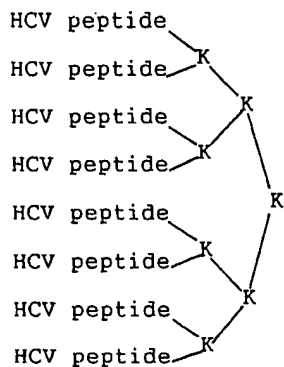
1 replaced by polystyrene beads, or multiple mini-columns filled  
2 with controlled pore size glass beads, or nitrocellulose paper  
3 strip, precoated with the peptides of the present invention for  
4 use as the solid phase immunosorbent.

5  
6 EXAMPLE 7

7 Immunization with Octameric HCV  
8 Peptides for the Elicitation of Sustaining High  
9 Titers of HCV Antibodies

10 In addition to the use of synthetic HCV peptides as  
11 immunogens for the generation of sequence-related anti-HCV  
12 antibodies for the ultimate development of an epitope-based  
13 subunit NANBH vaccine, another approach using a limited  
14 sequential propagation of a trifunctional amino acid lysine to  
15 form a core that serves as a low-molecular weight matrix  
16 carrier for peptide immunogens can also be applied. The  
17 trifunctional amino acid, Boc-Lys(Boc), is particularly  
18 suitable since both N- $\alpha$  and N- $\epsilon$  amino groups are available as  
19 reactive ends. Thus, sequential propagation of Boc-Lys(Boc)  
20 will generate  $2^n$  reactive ends. The first level coupling of  
21 Boc-Lys(Boc) will produce two reactive amino ends as a bivalent  
22 carrier. The sequential generations of a second and third step  
23 with Boc-Lys(Boc) will produce carriers containing  
24 four(tetra-valent), and eight (octa-valent) reactive amino ends  
25 to which peptide antigens are attached.

26 The HCV peptides as described in this invention can be  
27 incorporated onto this carrier system as illustrated below for  
28 the development of sustaining high titer HCV antibodies in  
29 mammals, including humans.  
30



Octameric HCV peptides of the present invention (Table 1) using the solid phase method of Merrifield are synthesized by an automated peptide synthesizer, either Applied Biosystems (ABI) Model 430A, or Biosearch Model 9500.

Both acid-labile tert-butyloxycarbonyl (t-Boc) and acid-stable groups are used for the protection of N- $\alpha$  amino acid and the functional side chains of the amino acids during the synthesis, respectively. The octameric peptides are synthesized by coupling onto a synthetic octamer resin.

An octamer resin is prepared by coupling di-t-Boc Lys onto 0.14 mmol/g MBHA (4-Methyl benzhydrylamine) resin. (Biosearch 9500 is used for this preparation due to its flexibility in scale). Di-Boc Lys single coupling is followed by two capping reactions (e.g. 0.3M Acetylimidazole in DMF dimethylformamide). The substitution level of synthetic octamer resin is determined by Ninhydrin Test.

Duncan Hartly random bred female guinea pigs (two per immunogen), weighing 400-500 gms, are used as the hosts. For initial immunizations, an aliquot of 100 ug octameric HCV peptide in 0.5 mL PBS is mixed with an equal volume of complete Freund's adjuvant and injected into each animal both

1 subcutaneously and intradermally over multiple sites. After  
2 two to three weeks of rest, an identical dosage of the same  
3 immunogen is given as a boost into each animal except that  
4 incomplete Freund's adjuvant is used. The animals are bled by  
5 heart puncture periodically to monitor each serum's anti-HCV  
6 titers. Subsequent booster shots are given periodically.

7  
8 EXAMPLE 8

9 Relative (%) Immunoreactivity for  
10 Synthetic Peptides By An  
11 Enzyme-Linked Immunosorbent Assay

12 Wells of 96-well plates were coated at 4°C overnight  
13 (or 1 hour at 37°C), with each of the additional nine peptides,  
14 VA, VB, VC, VD, VE (=V), VIA, VIB, VIC, VID, VIE (see Table 1  
15 for the above mentioned peptides), at 5 µg/mL at 100 µL per  
16 well in 10mM NaHCO<sub>3</sub> buffer, pH 9.5. Each peptide's  
17 immunoreactivity was measured as previously described in  
18 Example 1. Results obtained for the 10 peptides in the V and  
19 VI series are shown in Table 2. According to the EIA  
20 absorbance readings at 492nm (y axis) and the amino acid  
21 sequences for each of the corresponding HCV peptides (x axis),  
22 representative immunoreactivity profiles are plotted for four  
23 of the eight sera on the 10 peptides in the V and VI series,  
24 together with the first twenty peptides in the I, II and III  
25 series, as shown in Figures 1-1 to 1-4. Relative (%)  
26 immunoreactivity index for each of the additional 10 peptides  
27 is likewise calculated using peptide IIID as a reference.  
28 Additional clusters of residues, such as ASRQA and EVIAP, that  
29 are identified with these 10 peptides, were found to contribute  
30 additionally to the overall HCV antibody reactivity.

1 In summary, epitope mapping analysis conducted with a  
2 series of overlapping peptides reveals a varying degree of  
3 immunoreactivities between different HCV antibody positive  
4 samples and these HCV peptides. Based on the above-mentioned  
5 epitope mapping study, a third representative EIA using  
6 peptides IIF, IIID and V as the solid phase antigen was also  
7 configured for testing as shown in Example 9 in comparison to  
8 that using peptide IIF and IIID.

9  
10 EXAMPLE 9

11 Detection of Antibodies to HCV in Serial Samples  
12 by Enzyme-Linked Immunosorbent Assays

13 (a) A coded panel consisting of 24 samples derived  
14 from a case of transfusion transmitted NANBH were tested in two  
15 types of EIAs using plates coated with either a mixture of IIF  
16 and IIID at 5, 5 ug/mL or a mixture of IIF, IIID and V at 5,5,5  
17 ug/mL. The panel was provided by Dr. H. Alter of NIH and the  
18 results were decoded by his laboratory.

19 As shown in Figure 7-1, the two anti-HCV profiles, as  
20 tested by two formats, using Peptides IIF/IIID/V coated plate  
21 (Curve A) and Peptides IIF/IIID coated plate (curve B)  
22 respectively, spanning a ten year period revealed an  
23 interesting contrast.

24 According to the record, the seronegative patient  
25 received HCV contaminated blood units on August 20, 1980. As a  
26 result of the transfusion, a trace amount of passive HCV  
27 antibodies was detected in the recipient's serum by format A.  
28 Active development of HCV antibodies by the recipient became  
29 detectable by both formats from November 14th on (about three  
30 months after the initial transfusion). The HCV antibodies,

1 developed as a result of HCV infection through blood  
2 transfusion, persisted throughout the next ten years. Higher  
3 antibody signals were detected by plates coated with an extra  
4 peptide V (curve A) in sera collected four months after the  
5 transfusion. It appears that the epitope presented by peptide  
6 V, representing a neighboring immunodominant region, elicits  
7 abundant HCV antibodies at a slightly later stage than the  
8 epitopes represented by peptides IIF and IIID.

9 (b) Serial samples from one representative case of a  
10 hemodialysis patient with NANBH were provided by Dr. Cladd  
11 Stevens of New York Blood Center, N.Y., N.Y., and tested on  
12 plates coated with a mixture of three peptides, IIIF/IIID/V.  
13 The sample histories are shown in Figure 7-2. The results show  
14 that the peptide based EIA detects samples about two months  
15 after the onset of the acute phase of the disease as evidenced  
16 by the ALT elevation.

17 (c) Serial samples from a representative chimpanzee  
18 were tested with a peptide based HCV EIA using a mixture of  
19 IIIF/IIID/V peptides. This chimpanzee was inoculated on day 0  
20 with a well-characterized strain of NANBHIV. Following the  
21 acute phase of infection as evidenced by the rise of the ALT  
22 levels, antibodies to HCV were detected about 60 days after  
23 inoculation [Figure 7-3].

#### 24 EXAMPLE 10

##### 25 Screening of Low Risk Random Blood Donors 26 With the Peptide Based HCV EIA

27 2035 donor specimens obtained in a blood bank setting  
28 were tested by EIA coated with a mixture of Peptides IIF, IIID  
29 and V at 5 ug/mL each following the procedure described in  
30

1 Example 2. The results are shown in Figures 8-1 and 8-2. The  
2 frequency distribution of the peptide based HCV-EIA signal to  
3 cutoff ratios, suggested an initial reactive rate of 1.18% and  
4 a repeatably reactive rate of 1.08 respectively. 88% of the  
5 initial reactive specimens are repeatably reactive indicating a  
6 high reproducibility of the assay. The repeatably reactive  
7 rate of the peptide based HCV EIA obtained with the low risk  
8 random blood donor specimens, all volunteers, is lower than  
9 that obtained from the commercial paid donor population (See  
10 Example 2).

11  
12 EXAMPLE 11

13 Synthetic Peptide Based HCV Neutralization EIA  
14 As a Confirmatory Test

15 Wells of 96-well plates were coated at 4°C overnight  
16 (or for 1 hour at 37°C) with a mixture of two peptides IIF and  
17 IIFD at 5 ug/mL each in 100 uL 10mM NaHCO<sub>3</sub> buffer pH 9.5.  
18 Repeatably reactive specimens previously screened out by the  
19 direct HCV EIA were incubated with either a control specimen  
20 diluent buffer (i.e., PBS containing 20% by volume normal goat  
21 serum, 1% by weight gelatin and 0.05% by volume Tween 20) at a  
22 dilution of 1:20 volume to volume, or with the same specimen  
23 diluent buffer containing varying amounts of a HCV peptide  
24 analogue IV (see Table 1 for its amino acid sequence) and  
25 allowed to react for an hour at 37°.

26 200 uL of the peptide IV neutralized specimens were  
27 then added to each of the wells and allowed to react for 15  
28 minutes at 37°, followed by the EIA procedure as described in  
29 Example 2. Four representative reactive samples including two  
30 weakly reactives and two strongly reactives were tested. One

1 of the strong reactives was further diluted at 1:10 in the  
2 specimen diluent prior to neutralization testing. As shown in  
3 Figure 9 and Table 4, a dose dependent inhibition [or  
4 neutralization] of HCV EIA was observed with peptide IV. When  
5 compared with the controls, a significant inhibition was  
6 obtained with all four specimens even at a concentration of 50  
7 ug/mL peptide IV.

#### 8 EXAMPLE 12

##### 9 Detection of Antibodies to HCV in 10 Hemodialysis patients by EIA

11 A coded panel consisting of 74 samples from a group of  
12 hemodialysis patients was tested in two types of EIAs using  
13 plates coated with a mixture of HCV peptides IIH and V at 10,5  
14 ug/mL or a recombinant HCV protein based EIA. The panel was  
15 provided by investigators at the Japanese National Institute of  
16 Health and the results were decoded and compared to the  
17 recombinant HCV protein based EIA by the sera provider.

18 As shown in Figure 10, an x-y plot of the A492 nm  
19 readings for the peptide based HCV EIA and the recombinant HCV  
20 protein based HCV EIA revealed a high correlation between these  
21 two assays. (A cutoff value of 0.2 and 0.4 was obtained based  
22 on the corresponding assay design.) These 74 specimens  
23 obtained from the hemodialysis patients who are highly  
24 susceptible to HCV infection were grouped into four categories  
25 based on their respective reactivities with these two types of  
26 EIAs. The upper right block indicates samples that are scored  
27 positive by both assays, and the lower left block indicates  
28 samples that are scored negative by both assays. None of the  
29 74 high risk samples were found positive by the recombinant  
30

1 based EIA and negative by the peptide based EIA as shown in the  
2 upper left block; whereas five of these 74 high risk samples  
3 scored positive by the peptide based EIA and negative by the  
4 recombinant based EIA as shown in the lower right block  
5 indicating that the peptide based HCV EIA is more sensitive  
6 when tested with specimens derived from patients at high risks  
7 for HCV infection.

8  
9 EXAMPLE 13

10 Detection Of Anti-HCV Activity In Rare  
11 Specimens With An Elevated ALT Level

12 These results are representative of the acute phase of  
13 HCV infection by synthetic peptides of Peptide VII series,  
14 covering a region near the C-terminus of the HCV protein C-100,  
15 and Peptide IIH from the immunodominant region.

16 Wells or 96-well plates were coated at 37°C for 1 hour  
17 with each of the six peptides VIIA, VIIB, VIIC, VIID, IIG and  
18 IIH, at 5 ug/mL at 100 uL per well in 10mM NaHCO<sub>3</sub> buffer, pH  
19 9.5. Each peptide's immunoreactivity with the respective  
20 specimen was measured as previously described in Example 1. As  
21 shown in in Table 5, weak immunoreactivity was obtained with  
22 specimen 3 for peptides VIIC and VIID, but not VIIA and VIIB.  
23 Moderate immunoreactivity was obtained with specimen NAB-2-2  
24 for peptide IIH, but not IIG. Both specimens were found to  
25 have high ALT level and are representative of specimens from  
26 patients with acute phase of HCV infection.



Table 4

Peptide Based HCV Neutralization EIA  
As A Confirmatory Test

Specimens		Peptide IV Concentration									
		400 ug/mL		200 ug/mL		100 ug/mL		50 ug/mL		Control	
ID	Dilution	mA	%I	mA	%I	mA	%I	mA	%I	mA	%I
A	1:1	590	72.0	1129	46.5	1066	49.5	1363	35.4	2111	
		244	86.1	325	81.5	409	76.7	510	71.0	1762	
B	1:1	161	92.0	209	89.6	321	84.1	523	74.1	2021	
		117	89.0	162	84.7	155	85.4	153	85.6	1064	
C	1:1	27	89.8	23	91.3	38	85.7	34	87.2	266	

% INHIBITION =  $\frac{\text{mA}(\text{control}) - \text{mA}(\text{ug/mL})}{\text{mA}(\text{control})}$

Table 5

Code	Amino Acid Sequence	<u>A492nm</u>	
		Specimen No.	
		NAB-2-2	#3
IIH	SG, KPAIL, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMLA, EQFKQ, KALGL	1.232(+)	
IIG	II, PDREV, LYREF, DEMEE, CSQHL, PYIEQ, GMLA, EQFKQ, KALGL	0.013(-)	
VIIA	AVQWM, NRLIA, FASRG, NHVSP	0.109	
VIIB	RHV, GPGE, AVQWM, NRLIA, FASRG, NHVSP	0.224	
VIIC	V, VCAAI, LRRHV, GPGE, AVQWM, NRLIA, FASRG, NHVSP	0.674	
VIID	PGA, LVVGV, VCAAI, LRRHV, GPGE, AVQWM, NRLIA, FASRG, NHVSP	0.658	

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EXAMPLE 14

Measurement Of Relative (%) Immunoreactivity For Synthetic Peptide Covering An Immunodominant Region Of The Postulated HCV Core Protein By An Enzyme-Linked Immunosorbent Assay

Wells of 96-well plates were coated at 4°C overnight (or 1 hour at 37°C), with each of the ten peptides: VIIIA, VIIIB, VIIC, VIID, VIIIE(=VIII), IXA, IXB, IXC, IXD, and IXE(=IX), (see Table 7) prepared as described at 5 ug/mL at 100 uL per well in 10 mL NaHCO<sub>3</sub> buffer, pH 9.5. The rest of the plate coating and enzyme immunoassay procedures were performed exactly as described in Example 1.

Results obtained from this study are shown in Figs. 11-1 and 11-2. According to the EIA absorbance readings at 492 nm (y axis) and the amino acid sequences for each of the corresponding HCV peptides (x axis), representative immunoreactivity profiles are plotted for four of the eight panel sera as shown in Figures 11-1 and 11-2. Relative (%) immunoreactivity index for each of the 10 peptides (Table 7) is calculated against Peptide IIID (See Table 1), the one with the highest absorbance reading, based on the total absorbance of eight sera at 492 nm (See Tables 1 and 2 for examples of calculation). Figs. 11-1 and 11-2 show the amino acid sequences of the immunodominant region according to data obtained for immunoreactivity study. For example, serum sample 1 reacted strongly (ODs between 1.5 and 3.5) with peptides VIIIA and IXA, which are the smallest size in the 20mer range in the corresponding peptide series. Further addition of amino acids at the N-terminal end did not significantly enhance the immunoreactivity of these analogue peptide (see Figs. 11-1 and 11-2 for sample 1).

1           However, other serum samples such as #3 and #4 reacted  
2 much stronger with peptides VIIIB, VIIIC respectively and with  
3 an increasing immunoreactivity with the analogue peptides in  
4 the IX series (see Figs. 11-1 and 11-2, samples #3 and #4).  
5 Further, serum sample 2 reacted marginally with peptides in the  
6 corresponding VIII and IX series. These reactivity profiles  
7 indicate a more complicated epitope distribution along the  
8 postulated HCV core protein region and may include some  
9 discontinuous linear epitopes and conformational epitopes,  
10 requiring a longer size peptide to confer the best  
11 immunoreactivity for diagnostic purposes.

12           In summary, epitope mapping analysis conducted with a  
13 series of ten peptides covering an immunodominant region of the  
14 postulated HCV core protein, spanning a total of 119 amino acid  
15 residues as illustrated in Table 7 and Figs. 11-1 and 11-2,  
16 reveals varying degrees of immunoreactivity between different  
17 HCV antibody positive serum samples and analogue HCV Peptides  
18 of the VIII and IX series. In this case it is found preferably  
19 to have synthetic peptides with longer amino acid chains,  
20 ideally longer than 20, to optimally present these antigenic  
21 determinants to HCV antibodies.

22           Based on the above-mentioned epitope mapping study,  
23 additional representative EIAs using Peptides VIIIE, IXD, both  
24 derived from the HCV core region alone, or as a mixture with  
25 peptides IIH and V from the HCV nonstructural region were  
26 configured for the following studies described in Examples  
27 15, 16, 17 and 18.  
28  
29  
30

EXAMPLE 15

Detection Of Antibodies To HCV By Peptide Based Enzyme-Linked  
Immunosorbent Assay Using Format C, Format D, Format A

The following four groups of specimens:

- (a) individuals with AIDS, ARC(n=63);
- (b) individuals positive for HBsAg, (n=50);
- (c) individuals positive for antibodies to HBC protein, (n=22); and
- (d) individuals with elevated ( $>100$  i.u./L) alanine aminotransferase (ALT) enzyme activity, (n=86).

were analyzed on representative HCV peptide based EIAs according to the present invention, with the plates coated either with (i) peptides IIH and V at 5 and 3 ug/mL each (Format A), (ii) peptides IIH, V and VIIIE at 5, 3 and 2 ug/mL each (Format C, containing both the HCV core and nonstructural peptides) or (iii) Peptides VIIIE and IXD at 2 and 2 ug/mL each (Format D, HCV core peptides only).

Results obtained from the screening of a total of 221 well-characterized clinical specimens previously categorized into four groups, from (a) to (d) using a representative lot of peptide coated plates EIAs formatted as A, C or D were plotted on histograms as shown in Figs. 12-1, 12-2 and 12-3.

Out of a total of 63 AIDS/ARC patient samples analyzed, 46.0%, 55.6% and 50.8% of the patients were found to be HCV antibodies positive using EIA formats A, C and D respectively. Out of 50 HBsAg positive individuals, 36.0%, 42.0% and 36% of the individuals were found to also be HCV antibodies positive using EIA formats A, C and D respectively. Out of 22 HBC antibody positive individuals, 27.3%, 22.7%, and

1 18.2% were found to be HCV antibodies positive as detected by  
2 EIA formats A, C and D. Out of 86 patients with an elevated  
3 ALT levels, 90.7%, 91.5% and 85.4% were found to be HCV  
4 antibodies positive by EIA formats A, C and D. The overall  
5 signal to noise ratio distribution for the HCV positive samples  
6 were found to be higher with Formats C and D which included a  
7 peptide (VIIIIE) from the HCV core region than Format A which  
8 only employed peptides from the HCV nonstructural region as the  
9 solid phase antigen.

a 10 Except for one HBC antibody sample where the results  
11 is borderline positive (S/cutoff ratio ~1.0) with the HCV EIA  
12 Format A, Format C incorporating peptides (IIH, V and <sup>VIIIIE</sup>~~VIII~~)  
13 from both the HCV structural (core) and nonstructural regions  
14 was the most sensitive. The significant improvement in  
15 sensitivity makes Format C an ideal candidate for a HCV  
16 antibody screening assay.

17 EXAMPLE 16

18 Comparison Of Test Results Using The Three Peptide Based  
19 HCV EIA Formats (A, C And D) On Low Risk Random Blood Donors

20 Representative 264 donor specimens obtained in a blood  
21 bank setting were tested by all three EIA formats.

22 The results are shown in Figures 13-1 to 13-6. The  
23 frequency distributions of the peptide based HCV-EIA signal to  
24 cutoff ratios suggested an initial reactive rate of 1.13%, 3.0%  
25 and 3.0% with formats A, C and D respectively. The negative  
26 samples have a relative low signal to cutoff ratio in all three  
27 assay formats (see Figures 13-1, 13-3, and 13-5). Upon repeat  
28 testing, a repeatably reactive rate of 1.13%, 1.9% and 1.9%  
29 were obtained for formats A, C and D respectively. Among the  
30

1 sera identified as positives, there were four specimens which  
2 reacted strongly with both Formats C and D, but were identified  
3 as negatives by Format A. This indicates the possibility of  
4 false negative results when an HCV antibody detection assay  
5 does not include epitopes from the structural protein region.

#### 6 EXAMPLE 17

##### 7 Detection Of Antibodies To HCV In Well-Characterized 8 Serial Samples By Various Enzyme-Linked Immunosorbent Assays

9 (a) A coded panel consisting of 24 samples derived  
10 from a case of transfusion transmitted NANBH were tested in  
11 three HCV EIA formats (A, B and C) to determine the respective  
12 sensitivity of the formats in detecting seroconversion. The  
13 panel was provided by Dr. H. Alter of NIH and the results were  
14 decoded by his laboratory.

15 As shown in Figure 14-1, the three anti-HCV  
16 profiles, as tested by three formats using peptides IIF/IIID/V  
17 coated plate (Curve A); Peptides IIF/IIID coated plate (Curve  
18 B); and peptides IIH, V and VIIIE coated plate (Curve C)  
19 respectively, with sera spanning a ten year period revealed an  
20 interesting contrast.

21 As a result of the transfusion, a trace amount of  
22 passive HCV antibodies was detected in the recipient's serum by  
23 both format A, and C. Active development of HCV antibodies by  
24 the recipient became detectable by all three formats from  
25 November 14th on (about three months after the initial  
26 transfusion), with format C having the highest S/cutoff ratio  
27 on that bleed date. This finding further confirms the improved  
28 sensitivity obtained by using HCV EIA format C.  
29  
30

1 (b) Serial samples from a representative chimpanzee  
2 were tested with HCV EIA format C in comparison to a  
3 recombinant HCV C-100 based radioimmunoassay (RIA). The  
4 chimpanzee was inoculated on day 0 with a well-characterized  
5 strain of NANBHV. Following the acute phase of infection as  
6 evidenced by the rise of the ALT levels, antibodies to HCV were  
7 detected about 47 days after inoculation (Figure 14-2). HCV  
8 EIA Format C was able to detect HCV antibodies about 40 days  
9 early than the the rDNA based RIA. A higher signal to cutoff  
10 ratio was obtained with HCV EIA Format C than the rDNA RIA.

11 (c) Serial samples from three well-characterized  
12 representative HCV seroconversion panels, collected by  
13 Serologic Inc., were tested by HCV EIA formats A, C and D, as  
14 defined in Example 15 in addition to that previously tested  
15 with rDNA HCV C-100 based EIA. As shown in Table 8, both HCV  
16 EIA formats C and D were able to identify HCV antibody positive  
17 specimens in two out of three panels by four to eight weeks  
18 earlier than the rDNA HCV-100 based EIA and HCV EIA Format A.  
19 This further demonstrated the sensitivity of the HCV EIAs which  
20 incorporate peptides derived from the HCV structural (core)  
21 protein region.

#### 22 EXAMPLE 18

#### 23 Detection Of Antibodies To HCV In 24 Hemodialysis Patients By Various Forms Of HCV EIAs

25 A coded panel consisting of 74 samples from a group of  
26 hemodialysis patients was tested by three types of HCV EIAs; a  
27 recombinant HCV protein based EIA, and two using plates coated  
28 with either a mixture of HCV peptides IIH and V at 10, 5 ug/mL  
29 respectively (Format A), or a mixture of HCV peptides IIH, V  
30



1 and VIIIE at 5, 3 and 2 ug/mL respectively (Format C). The  
2 panel was provided by investigators at the Japanese National  
3 Institute of Health. Results were decoded and compared to the  
4 recombinant HCV protein based EIA by the sera provider.

5 As shown in Fig. 15-1, an x-y plot of the A492 nm  
6 readings for the peptide based HCV EIA Format C and the  
7 recombinant HCV protein based HCV EIA revealed an increased  
8 sensitivity with the peptide based HCV EIA format C when  
9 compared to the rDNA HCV C-100 protein based HCV EIA. (A  
10 cutoff value of 0.2 and 0.4 was obtained based on the  
11 corresponding assay design). These 74 specimens obtained from  
12 dialysis patients who are highly susceptible to HCV infection  
13 were grouped into four categories based on their respective  
14 reactivities with these two types of EIAs. The upper right  
15 block indicates samples that were scored positive by both  
16 assays, and the lower left block indicates samples that were  
17 scored negative by both assays. None of the 74 high risk  
18 samples were found positive by the recombinant based EIA and  
19 negative by the peptide based EIA as shown in the upper left  
20 block; whereas "eleven" of these 74 high risk samples scored  
21 positive by the peptide based EIA Format C and negative by the  
22 recombinant based EIA as shown in the lower right block.

23 An increase in sensitivity was obtained for the  
24 peptide based HCV EIA Format C (incorporating a HCV core  
25 peptide) when compared to HCV EIA Format A, which in turn  
26 showed an improved sensitivity compared with the recombinant  
27 HCV C-100 protein based EIA (see Example 12, Fig. 10).

28 To further document the validity of such a sensitivity  
29 comparison, other clinical data obtained for each of the  
30 dialysis patient specimens were tabulated along with the

1 corresponding EIA ratios (Table 9). Among the eleven marked  
2 specimens, most showed an increased level of GOT/GPT and were  
3 associated with frequent episodes of elevated GPT previously.  
4 All eleven specimens scored negative by the rDNA HCV C-100  
5 based EIA. However, these same samples reacted strongly (with  
a 12 6 O.D.  $\sim$  1.5) in the peptide based HCV EIA Format C. Since  
7 peptide VIII (=VIIIE) was synthesized according to amino acid  
8 sequences selected from the conserved structural (core) protein  
9 region, its inclusion in the peptide based HCV EIA (such as  
10 format C) will be particularly suitable when testing specimens  
11 from geographically distinct regions where a higher chance of  
12 strain-to-strain variation among the HCV isolates may be  
13 encountered.

14 It is to be understood that the above examples are  
15 illustrative of the present invention and are not meant to  
16 limit the scope thereof.

Table 8

Testing of Various Formats of HCV EIAs with Three Well-Characterized Seroconversion Panels

Panel	Donor #	Bleed Date	ALT $\frac{\text{I.U.}}{\text{L}}$	AST $\frac{\text{I.U.}}{\text{L}}$	rDNA HCV c-100	EIA Ratio		
						HCV EIA Format A (ns)	HCV EIA Format C (core+ns)	HCV EIA Format D (core)
Panel 1	02190D	880809	40.0	NA	0.03	0.093	0.108	0.205
		880816	32.0	NA	0.04	-0.014	0.045	0.129
		880823	32.0	NA	0.06	-0.050	0.025	0.072
		880830*	180.0	121.0	0.04	-0.050	1.037*	1.096*
		880928	401.0	352.0	0.19	0.100	7.193	7.703
		881109*	NA	NA	6.57*	16.700*	10.185	7.281
Panel 2	00269B	881122	NA	NA	6.57	16.671	9.770	9.321
		880815	39.0	NA	0.0	0.014	-0.058	-0.008
		880825	274.0	210.0	0.0	0.443	0.058	0.108
		880829	346.0	273.0	0.0	0.039	0.128	0.185
		880914	1175.0	722.7	6.5*	4.057	7.835*	5.984*
		881005	429.7	172.3	6.5	5.857	7.811	5.851
Panel 3	20830D	880829	63.0	65.0	0.04	-0.043	0.115	0.181
		880901*	81.0	NA	0.04	0.043	1.607*	1.108*
		880908	183.0	174.0	0.02	-0.043	2.506	3.116
		880928*	563.0	555.0	6.57*	3.800	9.827	9.659
		881026	436.0	151.0	6.57	13.786	10.630	10.566

Table 9

HCV Positivity in Serum Specimens  
Obtained from Japanese Dialysis Patients

Code No.	rDNA based HCA EIA OD Cutoff = 0.40	Peptide based HCV EIA Format A Cutoff = 0.205	Peptide based HCV EIA Format C Cutoff = 0.204	HBsAb	GOT/GPT Oct, 89	n: times during 1986-1988 when GPT was > 25 U/L
24	0.058	-0.001	0.005		2/3	0
25	0.042	0.005	0.007		9/9	0
26	0.105	-0.001	-0.003		4/4	0
27	1.837	1.469	2.312	-	3/6	2
28	1.797	1.637	2.398	-	20/21	2
29*	0.011	0.001	1.603		7/4	0
30	0.994	0.374	2.213		11/9	0
31	1.823	0.425	0.874	-	27/16	4
32	0.770	0.372	0.500	+	17/7	9
33	1.712	2.101	2.234	-	28/32	29
34	0.002	-0.003	0.007		11/14	0
35*	0.026	0.161	2.229	+	14/23	23
36*	0.065	0.018	2.286		20/18	
37	0.021	0.000	0.011	+	16/11	1
38	2.347	1.917	2.182	+	26/23	6
39	0.008	-0.007	0.004		7/6	0
40	0.026	0.006	-0.002		10/8	0
41*	0.061	0.118	1.933	+	9/6	
42	2.481	2.144	2.211	-	13/19	2
43	0.008	-0.005	-0.005	+	11/7	
44	0.009	-0.004	-0.005		4/4	0
45	0.009	0.000	-0.003		7/2	0
46	2.177	1.990	2.121	-	16/12	8
47	0.023	0.003	0.015		7/3	0
48	0.025	-0.003	0.002	+	18/11	
49	0.025	-0.001	-0.006		9/5	0
50	0.026	0.024	-0.003		9/3	
51	0.018	-0.003	-0.007	+	11/5	
52*	0.011	-0.003	1.366	-	33/52	29
53	2.251	1.296	2.218		8/7	0
54	0.050	0.017	0.040		10/7	0
55	0.020	-0.007	0.017	+	14/8	
56	0.033	-0.004	0.000		9/3	0
57	1.396	0.718	2.121	-	17/11	1
58	0.045	0.013	-0.003		13/12	
59	0.014	0.068	0.056		10/7	0
60	0.009	0.014	0.056	+	15/0	10
61	2.007	2.214	2.235	+	12/9	
62	0.171	0.001	0.003		11/7	0
63	1.121	0.529	2.383	+	18/10	
64	0.113	0.066	0.002		4/3	0
65	0.032	0.003	-0.003	+	7/5	3
66	0.039	-0.001	-0.002	+	11/6	
67*	0.049	0.037	2.119		16/11	

a

Code No.	rDNA based HCA EIA OD Cutoff = 0.40	Peptide based HCV EIA Format A Cutoff = 0.205	Peptide based HCV EIA Format C Cutoff = 0.204	HBsAb	GOT/GPT Oct, 89	n: times during 1986-1988 when GPT > 25 <del>u</del> <sup>u</sup> /L
68*	0.177	0.638	2.000	+	24/25	33
69	0.027	0.007	-0.007		6/3	0
70	0.031	-0.006	-0.001		16/9	0
71	0.781	0.473	2.151	+	13/8	14
72	0.110	0.002	0.059		13/8	0
73	0.043	-0.002	-0.007	-	2/3	0
74	0.014	0.001	-0.004		2/3	0
75	0.053	0.000	0.019	+	15/8	
76	0.060	0.015	0.018		14/7	0
77	0.011	0.001	-0.004		8/8	
78	0.042	0.002	0.023		3/0	0
79	0.537	0.219	1.742	+	11/7	
80	2.615	1.713	2.428	+	18/16	12
81	2.509	2.265	2.294		9/4	
82	0.019	0.000	0.120		11/5	0
83	0.511	1.928	2.229	-	19/11	5
84	0.020	0.016	0.095	-	12/9	
85	0.013	-0.003	0.116		10/7	0
86	0.003	-0.005	-0.006		19/5	
87	0.031	-0.009	0.009		10/6	0
88	0.039	0.019	0.004		6/2	0
89*	0.273	0.223	2.055	-	10/8	8
90	0.045	0.026	-0.002	-	7/3	3
91	0.018	0.003	-0.002		5/8	0
92	1.974	1.127	2.189	+	11/23	22
93	0.893	1.113	2.226	-	24/19	5
94*	0.267	0.353	2.029	-	18/12	1
95	0.026	-0.010	0.000	-	34/73	0
96*	0.021	0.002	1.599	-	13/30	27
97*	0.246	0.037	1.779		15/9	0
98	2.412	1.904	2.236	-	3/9	

WE CLAIM:

1. A peptide composition comprising a peptide with an amino acid sequence selected from the group consisting of:

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(x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

(IX)

wherein X is -OH or -NH<sub>2</sub>; and

(xi) analogues, segments, mixtures, combinations, conjugates and polymers thereof.

2. A peptide composition according to Claim 1 comprising a combination of Peptides I, II, III and V and having the amino acid sequence:

Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

3. A peptide composition according to Claim 1 comprising a segment of Peptide II and having an amino acid sequence selected from the group consisting of:

- (i) Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
- (ii) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
- (iii) Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
- (iv) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

*CS*  
*Sub B*  
(v) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

(vi) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

4. A peptide composition according to Claim 3 and having an amino acid sequence as follows:

*CS*  
*Sub B*  
Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

*Sub B*  
*CS*  
5. A peptide composition according to Claim 1 comprising a segment of Peptide III and having an amino acid sequence selected from the group consisting of:

(i) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

(ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

(iii) Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

(iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.



6. A peptide composition according to Claim 5 and having an amino acid sequence as follows:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

7. A peptide composition according to Claim 1 comprising a combination of Peptides VIII and IX and having the amino acid sequence:

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asp-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

8. A peptide composition according to Claim 1 comprising a segment of Peptide VIII and having an amino acid sequence selected from the group consisting of:

- (i) Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-X;
- (ii) Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-X;
- (iii) Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-X;
- (iv) Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

9. A peptide composition according to Claim 1 comprising a segment of Peptide IX and having an amino acid sequence selected from the group consisting of:

- (i) Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (ii) Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (iii) Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (iv) Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

10. A peptide composition according to Claim 1 wherein the peptide has an amino acid sequence as follows:

Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

11. A peptide composition according to Claim 1 wherein the peptide has an amino acid sequence as follows:

Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

12. A method of detecting antibodies to hepatitis C virus (HCV) or diagnosis of HCV infection or NANBH comprising the steps:

- (i) Preparing a peptide composition according to Claim 1;
- (ii) Using an effective amount of the peptide composition as an antigen to form a complex with antibodies to HCV or NANBH;
- (iii) Detecting the presence of the complex of peptide with antibodies to HCV or NANBH by an enzyme linked immunosorbent assay, an immunoradiometric assay or an agglutination assay or other immunoassays.

13. A method according to Claim 12 where in the peptide composition is coated on a solid substrate.

14. A method according to Claim 13 wherein the step of detecting the presence of the complex of peptide with antibodies to HCV or NANBH is by means of an enzyme linked immunosorbent assay

15. A method according to Claim 12 wherein the method of detecting the presence of the complex of peptide with antibodies to HCV or NANBH is by using an immunoradiometric-assay.

16. A method according to Claim 12 wherein the method of detecting the presence of the complex of peptide with antibodies to HCV or NANBH is by an agglutination assay.

17. A method according to Claim 12 wherein the peptide composition is a combination of peptides I, II, III and V and having the amino acid sequence:

Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-  
Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-  
Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-  
Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-  
Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-  
Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-  
Lys-His-Met-Trp-Asn-Phe-X

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

18. A method according to claim 12 wherein the peptide composition comprises a segment of Peptide II and has an amino acid sequence selected from the group consisting of:

- (i) Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
- (ii) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
- (iii) Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
- (iv) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
- (v) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
- (vi) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

19. A method according to claim 12, wherein the peptide composition comprises a peptide having an amino acid sequence:

Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

20. A method according to claim 12 wherein the peptide composition is a segment of peptide III and having an amino acid sequence selected from the group consisting of:

- (i) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;
- (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;
- (iii) Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;
- (iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

21. A method according to claim 12 wherein the peptide composition comprises a peptide having an amino acid sequence:

- (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

22. A method according to Claim 12 wherein the peptide composition comprises a combination of Peptides VIII and IX and has an amino acid sequence:

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

23. A method according to Claim 12 wherein the peptide composition comprises a segment of Peptide VIII and has an amino acid sequence:

Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

24. A method according to Claim 12 wherein the peptide composition comprises a segment of Peptide IX and has an amino acid sequence selected from the group consisting of:

- (i) Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (ii) Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (iii) Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

(iv) Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

25. A method according to Claim 12 wherein the peptide composition comprises a peptide having an amino acid sequence as follows:

Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

26. A method according to Claim 12 wherein the peptide composition comprises a peptide having an amino acid sequence as follows:

Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

27. Antibodies to HCV or NANBHV produced by using as an immunogen a peptide composition comprising a peptide with an amino acid sequence selected from the group consisting of:

(i) Glu-Glu-Ser-Cys-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X (I)

(ii) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (II)

- (iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)
- (iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X (III)
- (v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X (IV)
- (vi) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X (V)
- (vii) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X (VI)
- (viii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X (VII)
- (ix) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-His-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X, and (VIII)
- (x) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X (IX)

wherein X is -OH or -NH<sub>2</sub>; and

- (xi) analogues, segments, mixtures, combinations, conjugates and polymers thereof.

28. An enzyme linked immunosorbent assay (ELISA) test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:



- Sub B5*
- (i) compartmented enclosure containing multiple wells coated with a peptide composition according to Claim 1;
  - (ii) a negative control sample;
  - (iii) an inactivated HCV positive control sample;
  - (iv) specimen diluent comprising PBS buffer containing 20% by volume normal goat serum; 1% by weight gelatin and 0.05% by weight TWEEN 20;
  - (v) peroxidase labelled antibodies to human IgG; and
  - (vi) a color change indicator.

29. An ELISA test kit according to claim 28 wherein the multiple wells are coated with a peptide composition which is a combination of Peptide I, II, III and V with an amino acid sequence:

Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-  
Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-  
Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-  
Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-  
Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Asp-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-  
Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-  
Lys-His-Met-Trp-Asn-Phe-X

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

*D5* *Sub B5*  
30. An ELISA test kit according to claim 28 wherein the multiple wells are coated with a peptide composition comprising a segment of Peptide II and having an amino acid sequence selected from the group consisting of:

- (i) Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
- (ii) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
- (iii) Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

- Sub  
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D5
- (iv) ~~Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;~~
- (v) ~~Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;~~
- (vi) ~~Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;~~

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

31. An ELISA test kit according to claim 28 wherein the multiple wells are coated with a peptide having an amino acid sequence:

~~Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;~~

wherein X is OH or -NH<sub>2</sub> and analogues thereof.

32. An ELISA test kit according to claim 28 wherein the multiple wells are coated with a peptide composition comprising a segment of Peptide III having an amino acid sequence selected from the group consisting of:

- (i) ~~Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;~~
- (ii) ~~Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;~~
- (iii) ~~Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;~~
- (iv) ~~Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;~~

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

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33. An ELISA test kit according to claim 32 wherein the peptide is:

- (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

34. An ELISA test kit according to Claim 28 wherein the multiple wells are coated with a peptide composition comprising a combination of Peptide VIII and IX having an amino acid sequence:

Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-Gln-Pro-Ala-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

Sub 16  
35. An ELISA test kit according to Claim 28 wherein the multiple wells are coated with a peptide composition comprising a segment of Peptide VIII having an amino acid sequence selected from the group consisting of:

- (i) Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;
- (ii) Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;
- (iii) Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;

Sub  
C6

(iv) Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

16. An ELISA test kit according to Claim 28 wherein the multiple wells are coated with a peptide composition comprising a segment of Peptide IX having an amino acid sequence selected from the group consisting of:

- (i) Ile-Pro-Lys-Val-Arg-Arg-Pro-Gln-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (ii) Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (iii) Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (iv) Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

37. An ELISA test kit according to Claim 28 wherein the multiple wells are coated with a peptide composition comprising a peptide having an amino acid sequence as follows:

Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

38. An ELISA test kit according to Claim 28 wherein the multiple wells are coated with a peptide composition comprising a peptide having an amino acid sequence as follows:

Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

39. An ELISA test kit according to Claim 28 wherein the multiple wells are coated with a peptide composition comprising a mixture of Peptides IIH and V, Peptides IIH and V having the following amino acid sequences respectively:

- (i) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)
- (ii) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X; (V)

wherein X is -OH or -NH<sub>2</sub> or analogues thereof.

40. An ELISA test kit according to Claim 28 wherein the multiple wells are coated with a peptide composition comprising a mixture of Peptides IIF, IIID and V, Peptide IIF, IIID and V having the following amino acid sequences respectively:

- (i) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X; (IIF)
- (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X; (IIID)

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(iii) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X;

(V)

wherein X is -OH or -NH<sub>2</sub> or analogues thereof.

41. An ELISA test kit according to Claim 28 wherein the multiple wells are coated with a peptide composition comprising a mixture of Peptides IIH, V and VIIIE, Peptide IIH, V and VIIIE having the following amino acid sequences respectively:

(i) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

(IIH)

(ii) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X;

(V)

(iii) Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;

(VIIIE)

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

42. An ELISA test kit according to Claim 28 wherein the multiple wells are coated with a peptide composition comprising a mixture of Peptides VIIIE and IXD, Peptide VIIIE and IXD having the following amino acid sequences respectively:

(i) Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;

(VIIIE)

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D6

(ii) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-  
Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-  
Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-  
Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-  
Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-  
Gly-X;

(IXD)

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

43. A peptide which is immunoreactive to HCV with at least about 15 to not more than about 65 amino acids in a sequences having as a segment thereof said segment selected from the group consisting of:

- (i) Ser-Gly-Lys-Pro-Ala;  
(ii) Ile-Ile-Pro-Asp-Arg;  
(iii) Glu-Glu-Cys-Ser-Gln;  
(iv) His-Leu-Pro-Tyr-Ile;  
(v) Glu-Gln-Gly-Met-Met;  
(vi) Leu-Ala-Glu-Gln-Phe;  
(vii) Lys-Gln-Lys-Ala-Leu;  
(viii) Gly-Leu-Leu-Gln-Thr;  
(ix) Glu-Val-Ile-Ala-Pro; and  
(x) Glu-Val-Leu-Tyr-Arg.
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B6

add B7

add  
C6

INVENT

Docket No. 1151-4043

COMBINED DECLARATION AND POWER OF ATTORNEY FOR  
ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL,  
DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV, DIAGNOSIS OF HCV INFECTION AND PREVENTION THEREOF AS VACCINES  
the specification of which

a. ☒ is attached hereto

b. ☐ was filed on \_\_\_\_\_ as application Serial No. \_\_\_\_\_  
and was amended on \_\_\_\_\_. (if applicable).

PCT FILED APPLICATION ENTERING NATIONAL STAGE

c. ☐ was described and claimed in International Application  
No. \_\_\_\_\_ filed on \_\_\_\_\_ and as amended  
on \_\_\_\_\_. (if any).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

☐ I hereby claim foreign priority benefits under Title 35, United States Code § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

☐ The attached 35 U.S.C. § 119 claim for priority for the U.S. application(s) listed below forms a part of this declaration.

Country	Application Number	Date of filing (day, month, yr)	Date of issue (day, month, yr)	Priority Claimed
_____	_____	_____	_____	<input type="checkbox"/> YES <input type="checkbox"/> NO
_____	_____	_____	_____	<input type="checkbox"/> YES <input type="checkbox"/> NO
_____	_____	_____	_____	<input type="checkbox"/> YES <input type="checkbox"/> NO



ENT

Docket No. 1151-4043

ADDITIONAL STATEMENTS FOR  
DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART

I hereby claim the benefit under Title 35, United States Code § 120 of any United States application(s) listed below.

<u>07/481,348</u>	<u>February 16, 1990</u>	<u>pending</u>
Application Serial No.	Filing Date	Status (patented, pending, abandoned)
<u>07/510,153</u>	<u>April 16, 1990</u>	<u>pending</u>
Application Serial No.	Filing Date	Status (patented, pending, abandoned)

[X] In this continuation-in-part application, insofar as the subject matter of any of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint the following attorneys and/or agents with full power of substitution and revocation, to prosecute this application, to receive the patent, and to transact all business in the Patent and Trademark Office connected therewith: Jerome G. Lee (Reg. No. 16,967), John D. Foley (Reg. No. 16,836), John A. Diaz (Reg. No. 19,550), Thomas P. Dowling (Reg. No. 19,221), John C. Vassil (Reg. No. 19,098), Warren H. Rotert (Reg. No. 19,659), Alfred P. Ewert (Reg. No. 19,887), David H. Pfeffer, P.C. (Reg. No. 19,825), Harry C. Marcus (Reg. No. 22,390), Robert E. Paulson (Reg. No. 21,046), Stephen R. Smith (Reg. No. 22,615), Kurt E. Richter (Reg. No. 24,052), J. Robert Dailey (Reg. No. 27,434), Eugene Moroz (Reg. No. 25,237), John F. Sweeney (Reg. No. 27,471), Arnold I. Rady (Reg. No. 26,601), Christopher A. Hughes (Reg. No. 26,914), William S. Feiler (Reg. No. 26,728), Joseph A. Calvaruso (Reg. No. 28,287), James W. Gould (Reg. No. 28,859), Richard C. Komson (Reg. No. 27,913), Israel Blum (Reg. No. 26,710) and Bartholomew Verdirame (Reg. No. 28,483) of Morgan & Finnegan whose address is: 345 Park Avenue, New York, New York 10154.

[X] I hereby authorize the U.S. attorneys and/or agents named hereinabove to accept and follow instructions from Maria C.H. Line, Reg. No. 29,323

as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. attorneys and/or agents and me. In the event of a change in the person(s) from whom instructions may be taken I will so notify the U.S. attorneys and/or agents named hereinabove.

AGENT

Docket No. 1151-4043

I hereby specify the following as the correspondence address to which all communications about this application are to be directed:

Maria C.H. Lin

SEND CORRESPONDENCE TO:

MORGAN & FINNEGAN, 345 Park Avenue, New York, New York 10154

DIRECT TELEPHONE CALLS TO: Maria C.H. Lin  
(212) 758-4800 or direct dial

(212) 415-8745

40100  
Full name of sole or first inventor Chang Yi Wang

Inventor's signature\* Chang Yi Wang July 25, 1990

Residence 159 Hill Park Avenue, Great Neck, New York 11021 NY

Citizenship U.S.A.

Post Office Address United Biomedical Inc., 2 Nevada Drive, Lake Success, NY 11042

Full name of second joint inventor, if any \_\_\_\_\_

Inventor's signature\* \_\_\_\_\_

Residence \_\_\_\_\_ date \_\_\_\_\_

Citizenship \_\_\_\_\_

Post Office Address \_\_\_\_\_

[ ] ATTACHED IS ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY THIRD AND SUBSEQUENT INVENTORS FORM.

\* Before signing this declaration, each person signing must:

1. Review the declaration and verify the correctness of all information therein; and
2. Review the specification and the claims, including any amendments made to the claims.

After the declaration is signed, the specification and claims are not to be altered.

To the inventor(s):

The following are cited in or pertinent to the declaration attached to the accompanying application:

Title 37, Code of F. a) Regulation, §1.56

Duty of disclosure....

(a) A duty of candor and good faith toward the Patent and Trademark Office rests on the inventor, on each attorney or agent who prepares or prosecutes the application and on every other individual who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application. All such individuals have a duty to disclose to the Office information they are aware of which is material to the examination of the application. Such information is material where there is a substantial likelihood that a reasonable examiner would consider it important in deciding whether to allow the application to issue as a patent. The duty is commensurate with the degree of involvement in the preparation or prosecution of the application.

\* \* \* \*

c) Any application may be stricken from the files if:

- (1) An oath or declaration ... is signed in blank;
- (2) An oath or declaration ... is signed without review thereof by the person making the oath or declaration;
- (3) an oath or declaration ... is signed without review of the specification, including the claims ...;

or

- (4) The application papers filed in the Office are altered after the signing of an oath or declaration ... referring to those application papers.

Title 35, U.S. Code, § 119

Benefit of earlier filing date in foreign country; right of priority

An application for patent for an invention filed in this country by any person who has, or whose legal representatives or assigns have, previously regularly filed an application for a patent for the same invention in a foreign country which affords similar privileges in the case of applications filed in the United States or to citizens of the United States, shall have the same effect as the same application would have if filed in this country on the date on which the application for patent for the same invention was first filed in such foreign country, if the application in this country is filed within twelve months from the earliest date on which such foreign application was filed; but no patent shall be granted on any application for patent for an invention which had been patented or described in a printed publication in any country more than one year before the date of the actual filing of the application in this country, or which had been in public use or on sale in this country more than one year prior to such filing.

Title 35, U.S. Code, § 102

Benefit of earlier filing date in the United States

An application for patent for an invention disclosed in the manner provided by the first paragraph of section 112 of this title in an application previously filed in the United States, or as provided by section 363 of this title, which is filed by an inventor or inventors named in the previously filed application shall have the same

effect, as to such invention, as though filed on the date of the prior application, if filed before the patenting or abandonment of or termination of proceedings on the first application or an application similarly entitled to the benefit of the filing date of the first application and if it contains or is amended to contain a specific reference to the earlier filed application.

#### Title 35, U.S. Code § 101

##### Inventions patentable

Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

#### Title 35 U.S. Code § 102

##### Conditions for patentability; novelty and loss of right to patent

A person shall be entitled to a patent unless —

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this country, more than one year prior to the date of the application for patent in the United States, or

(b) the invention was patented or described in a printed publication in this or foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States, or

(c) he has abandoned the invention, or

(d) the invention was first patented or caused to be patented, or was the subject of an inventor's certificate, by the applicant or his legal representatives or assigns in a foreign country prior to the date of the application for patent in this country on an application for patent or inventor's certificate filed more than twelve months before the filing of the application in the United States, or

\* \* \* \*

(e) the invention was described in a patent granted on an application for patent by another filed in the United States before the invention thereof by the applicant for patent, or on an international application by another who has fulfilled the requirements of paragraphs (1), (2), and (4) of section 371(c) of this title before the invention thereof by the applicant for patent, or

(f) he did not himself invent the subject matter sought to be patented, or

(g) before the applicant's invention thereof the invention was made in this country by another who had not abandoned, suppressed, or concealed it. In determining priority of invention there shall be considered not only the respective dates of conception and reduction to practice of the invention, but also the reasonable diligence of one who was first to conceive and last to reduce to practice, from a time prior to conception by the other ...

#### Title 35, U.S. Code § 103

##### Conditions for patentability; non-obvious subject matter

A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter

as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Subject matter developed by another person, which qualifies as prior art only under subsection (f) or (g) of section 102 of this title, shall not preclude patentability under this section where the subject matter and the claimed invention were, at the time the invention was made, owned by the same person or subject to an obligation of assignment to the same person.

Title 35, U.S. Code § 112 (in part)

Specification

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Please read carefully before signing the Declaration attached to the accompanying Application.

If you have any questions, please contact Morgan & Finnegan



PATENT

Docket No. 1151-4043

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) or Patentee(s): Chang Yi Wang

Group Art Unit: To be assigned

Serial No. or Patent No.: To be assigned

Examiner: To be assigned

Filed or Issued: July 25, 1990

For: SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV,  
DIAGNOSIS OF HCV INFECTION AND PREVENTION THEREOF AS VACCINES  
VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY  
STATUS (37 CFR § 1.9 (f) and § 1.27 (c)) - SMALL BUSINESS CONCERN

I hereby declare that I am

☐ the owner of the small business concern identified below:

☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN United Biomedical Inc.

ADDRESS OF CONCERN 2 Nevada Drive, Lake Success, New York 11042

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR §§ 121.3-18, and reproduced in 37 CFR § 1.9(d), for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled: SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV, DIAGNOSIS OF HCV INFECTION AND PREVENTION THEREOF AS VACCINES by inventor(s) Chang Yi Wang

described in

☒ the specification filed herewith

☐ application Serial No. \_\_\_\_\_, filed \_\_\_\_\_

☐ Patent No. \_\_\_\_\_, issued \_\_\_\_\_

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR § 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR § 1.9(d) or a nonprofit organization under 37 CFR § 1.9(e).

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR § 1.27)

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_  
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

NAME \_\_\_\_\_

ADDRESS \_\_\_\_\_  
☐ Individual ☐ Small Business Concern ☐ Nonprofit Organization

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or

Express mail No. LB211744522

at the time of paying, the smallest of the issue fee or any maintenance fee due after the date of filing, which status as a small entity is no longer appropriate. (37 CFR § 1.28(b))

17/559799

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING Chang Yi Wang

TITLE OF PERSON OTHER THAN OWNER Chief Executive Officer

ADDRESS OF PERSON SIGNING 2 Nevada Drive, Lake Success, New York 11042

SIGNATURE

Chang Yi Wang

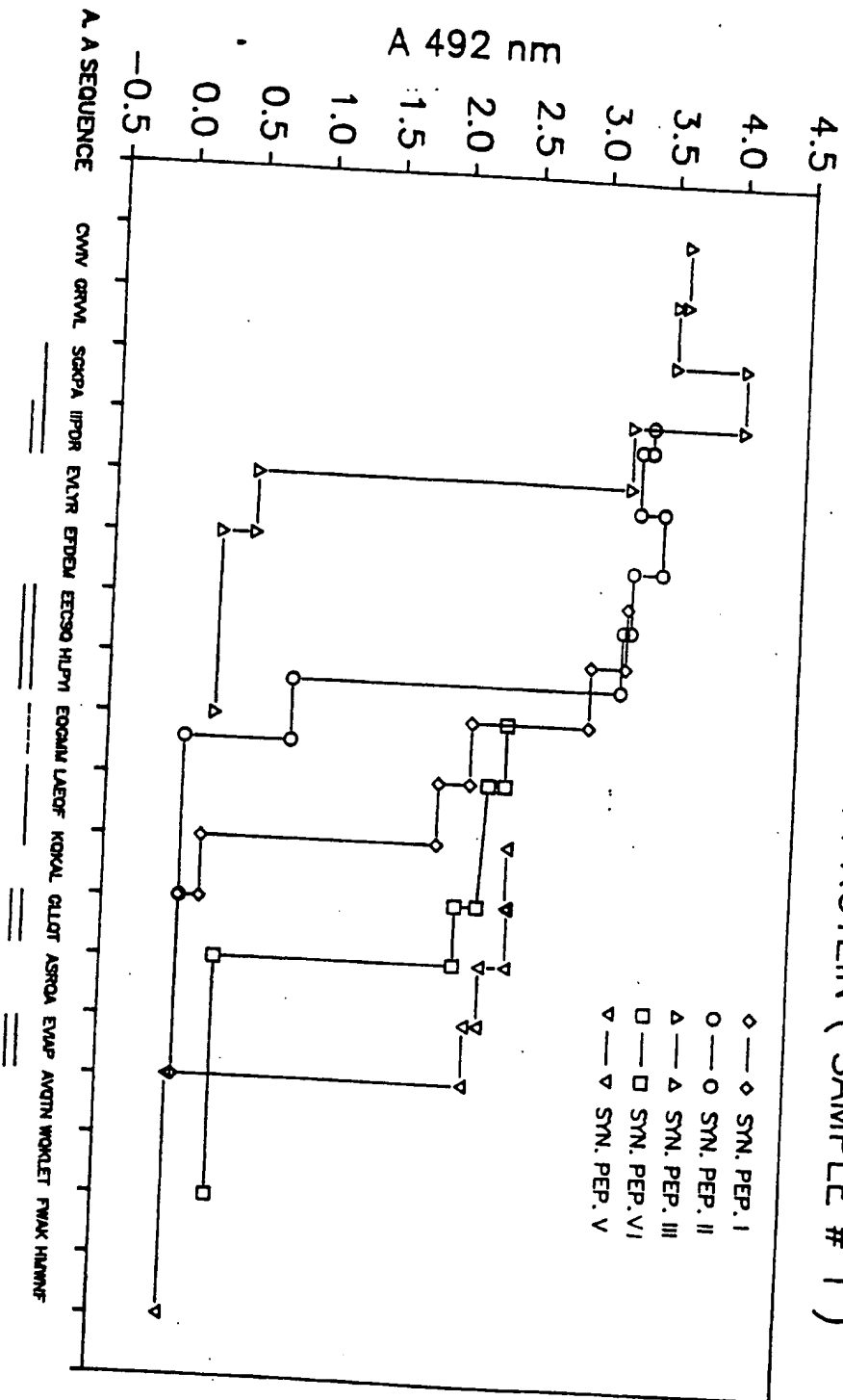
DATE

July 25, 1990

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AS ORIGINAL FILED

07/558799

*Fig 1-1*  
EPI TOPE MAPPING OF AN IMMUNODOMINANT REGION OF  
THE HCV SOD-C100 FUSION PROTEIN ( SAMPLE # 1 )



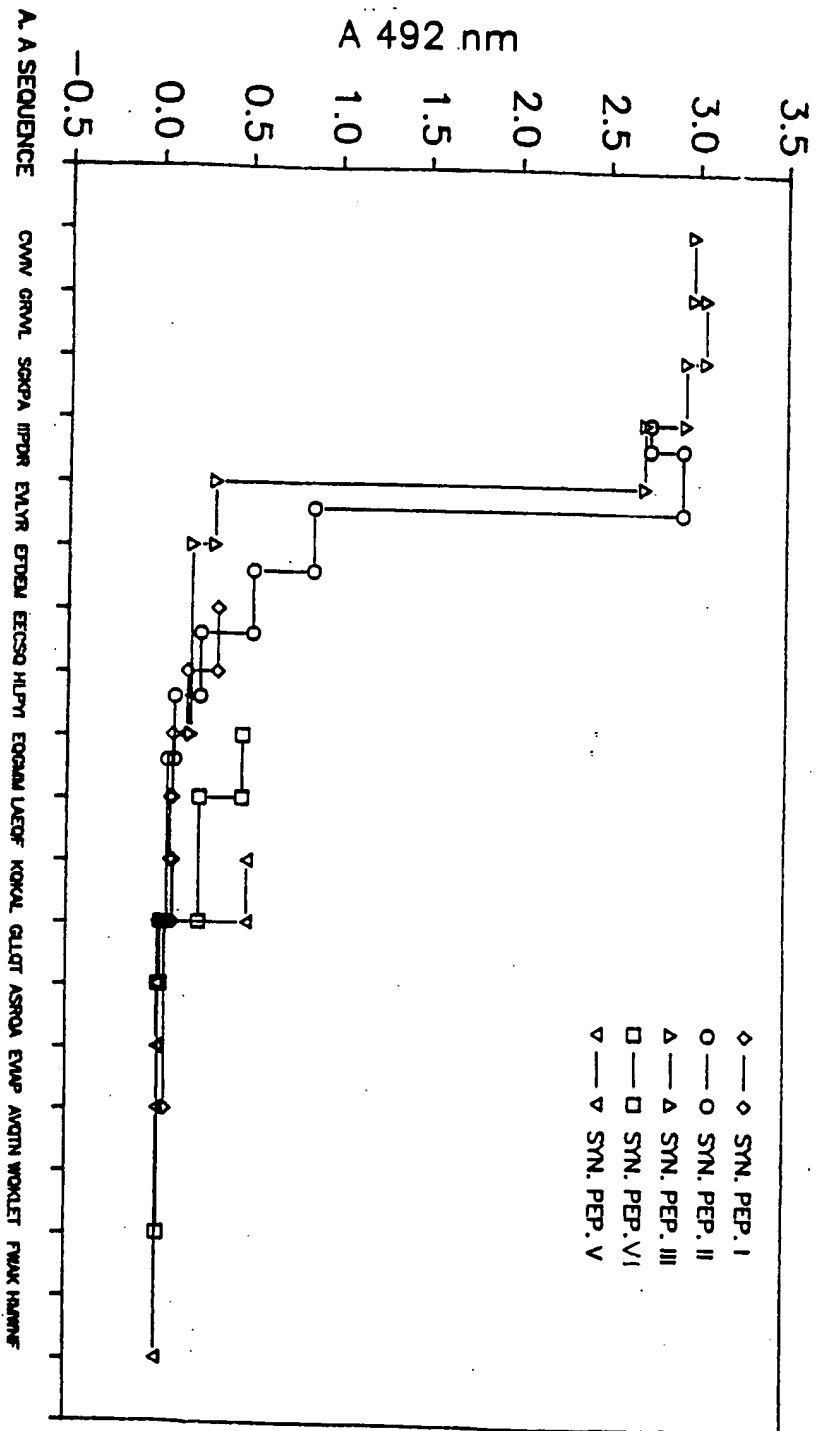


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Fig 1-2

# EPITOPE MAPPING OF AN IMMUNODOMINANT REGION OF THE HCV SOD-C100 FUSION PROTEIN (SAMPLE # 2)

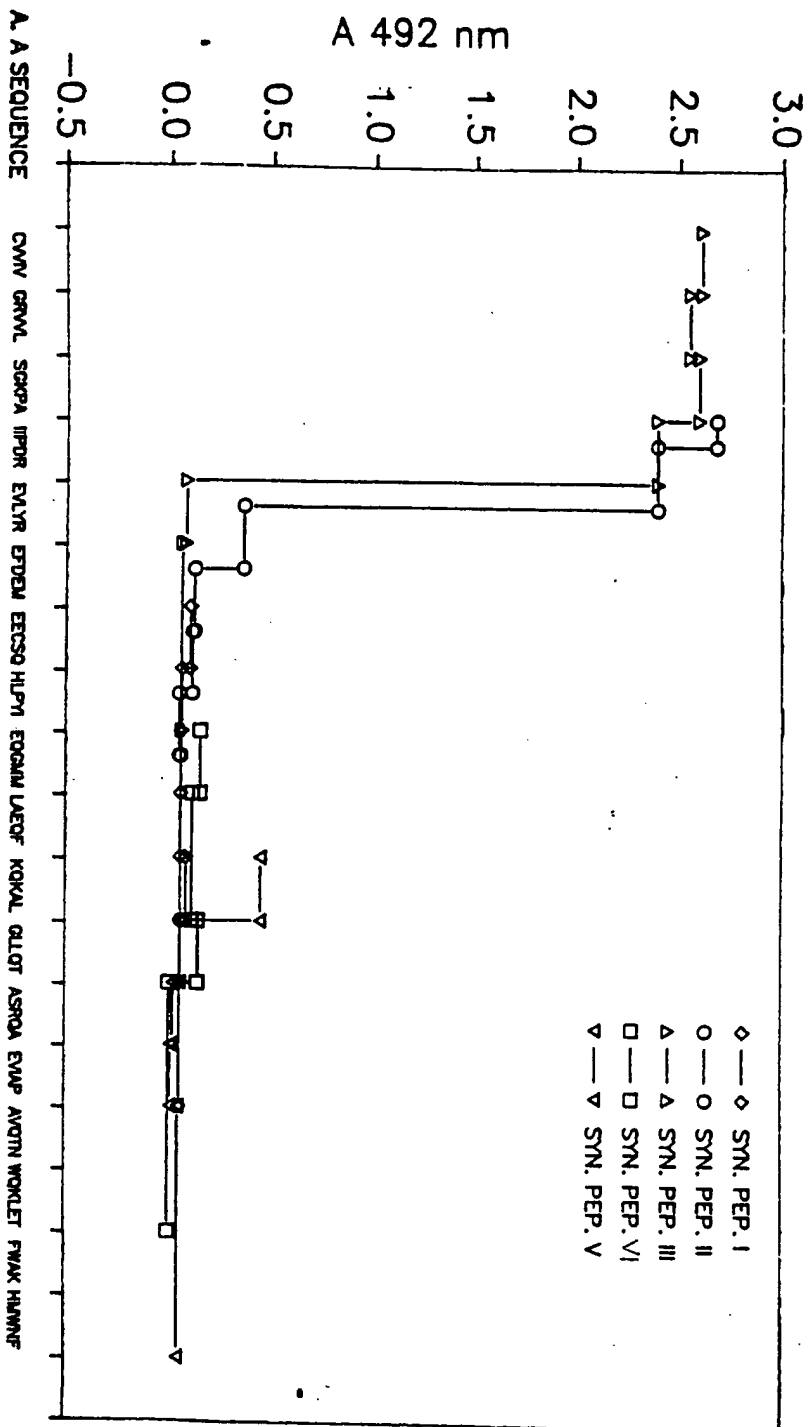


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11/558799

Fig 1-3

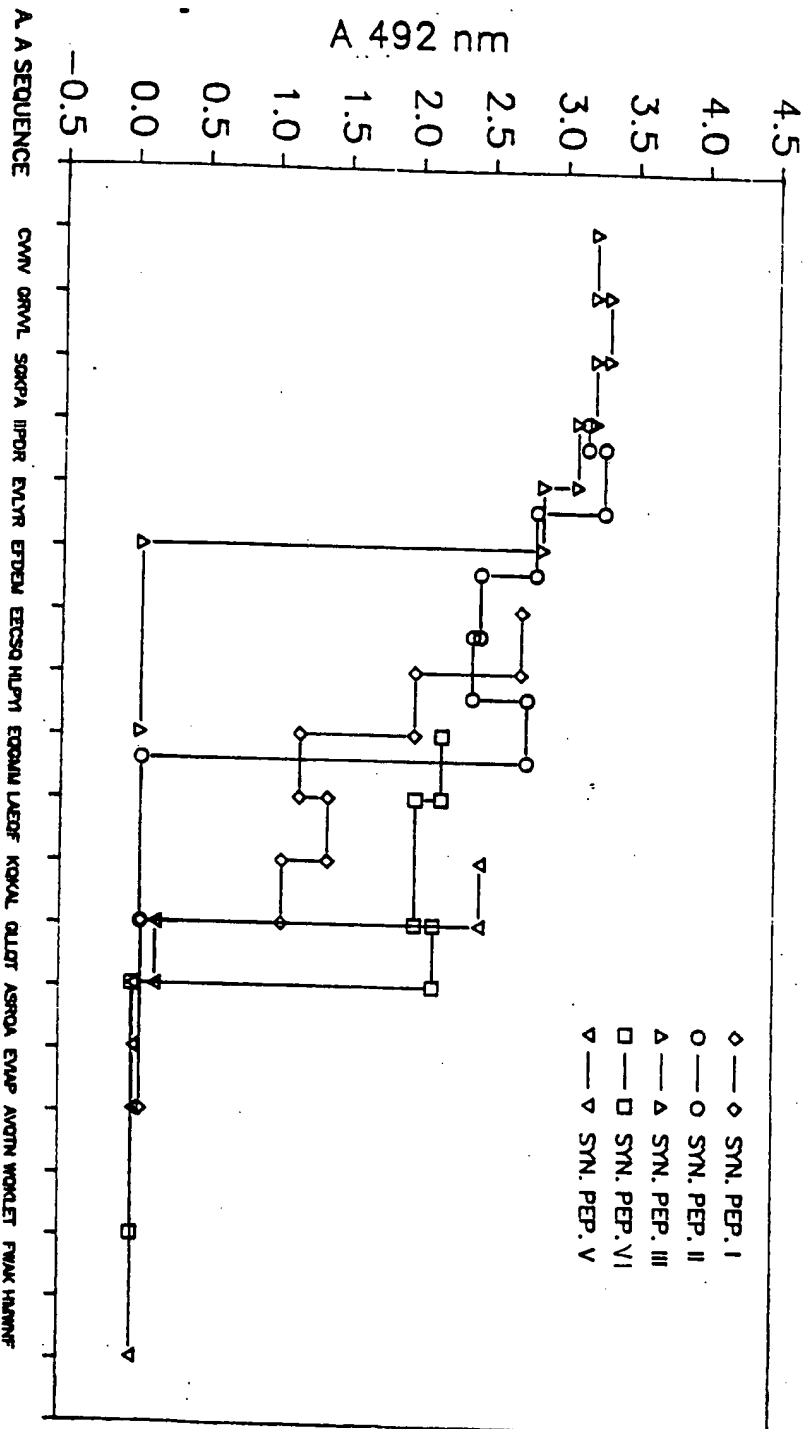
# EPITOPE MAPPING OF AN IMMUNODOMINANT REGION OF THE HCV SOD-C100 FUSION PROTEIN ( SAMPLE # 3 )



87-559799

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AS ORIGINAL FILED

*Fig 1-4*  
EPIOTOPE MAPPING OF AN IMMUNODOMINANT REGION OF  
THE HCV SOD-C100 FUSION PROTEIN ( SAMPLE # 4 )

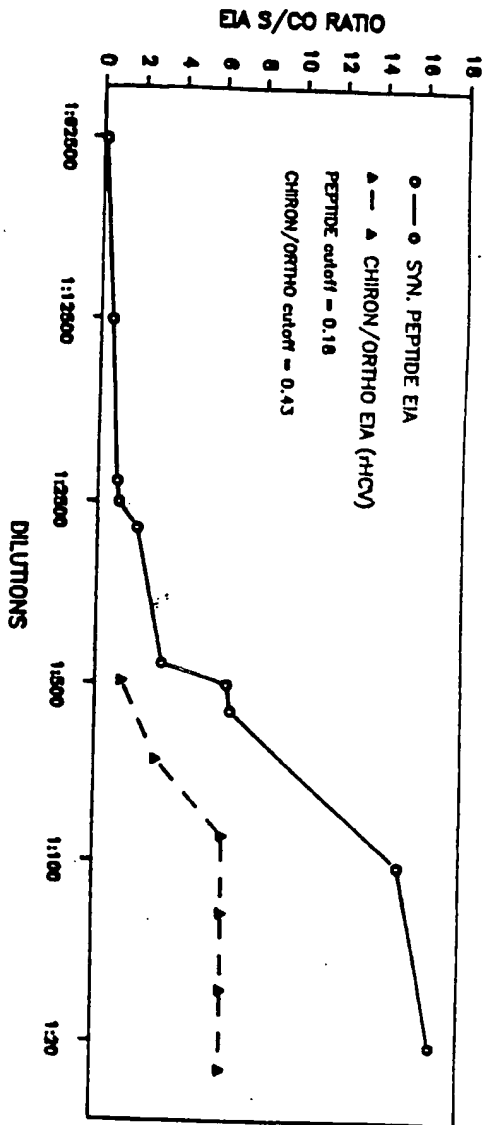


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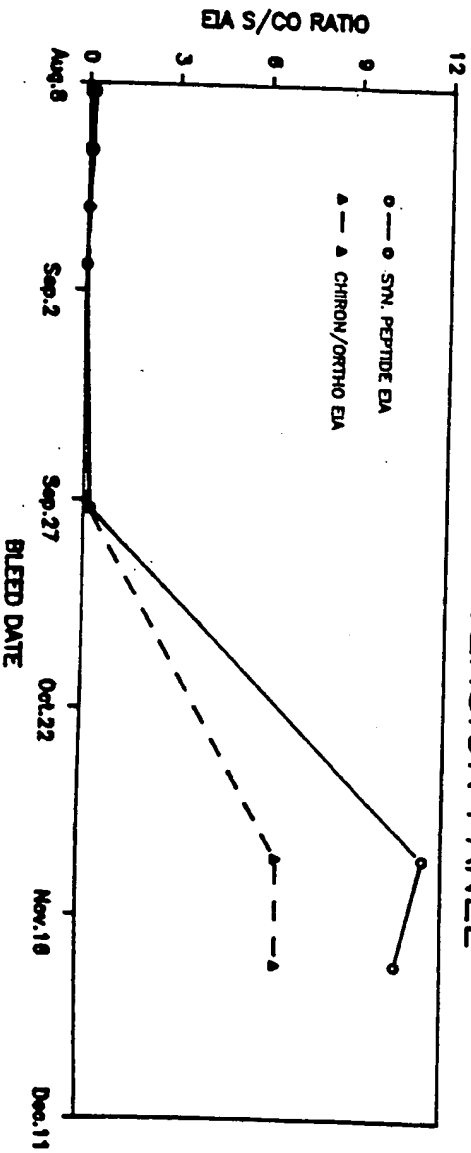
Fig 2-1

Fig 2-2

# COMPARISON of SYN. PEPTIDE VS rDNA PRODUCED HCV EIA



## HCV SEROCONVERSION PANEL



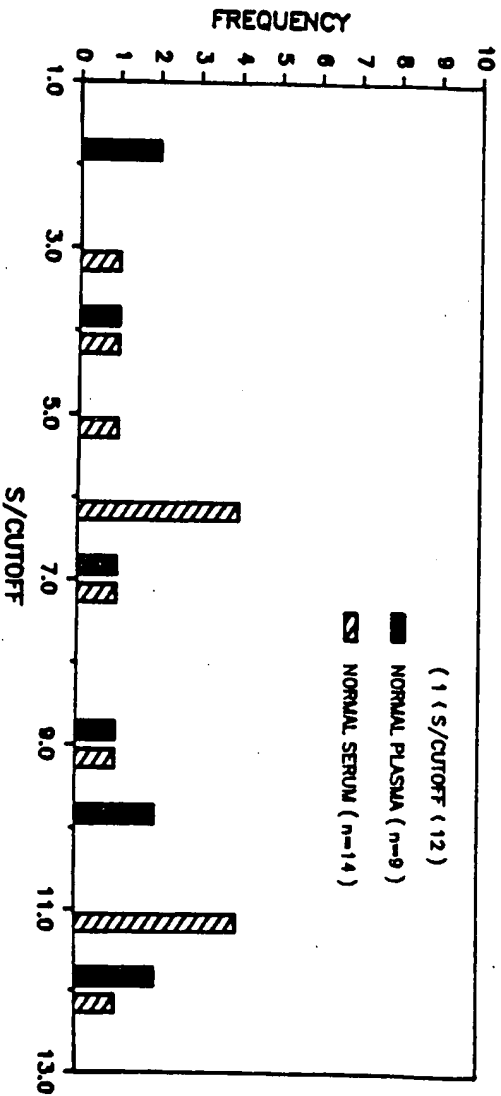
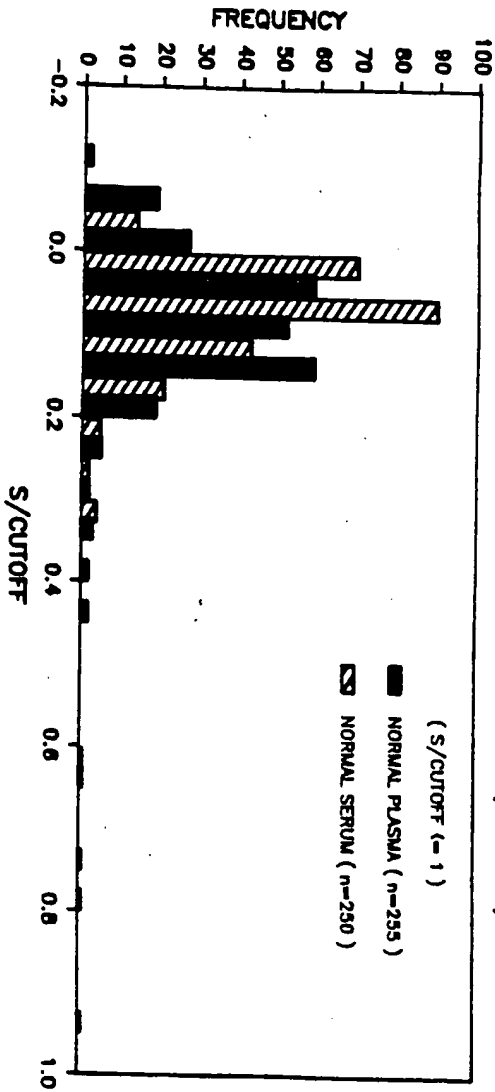
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Fig 3-1

Fig 3-2

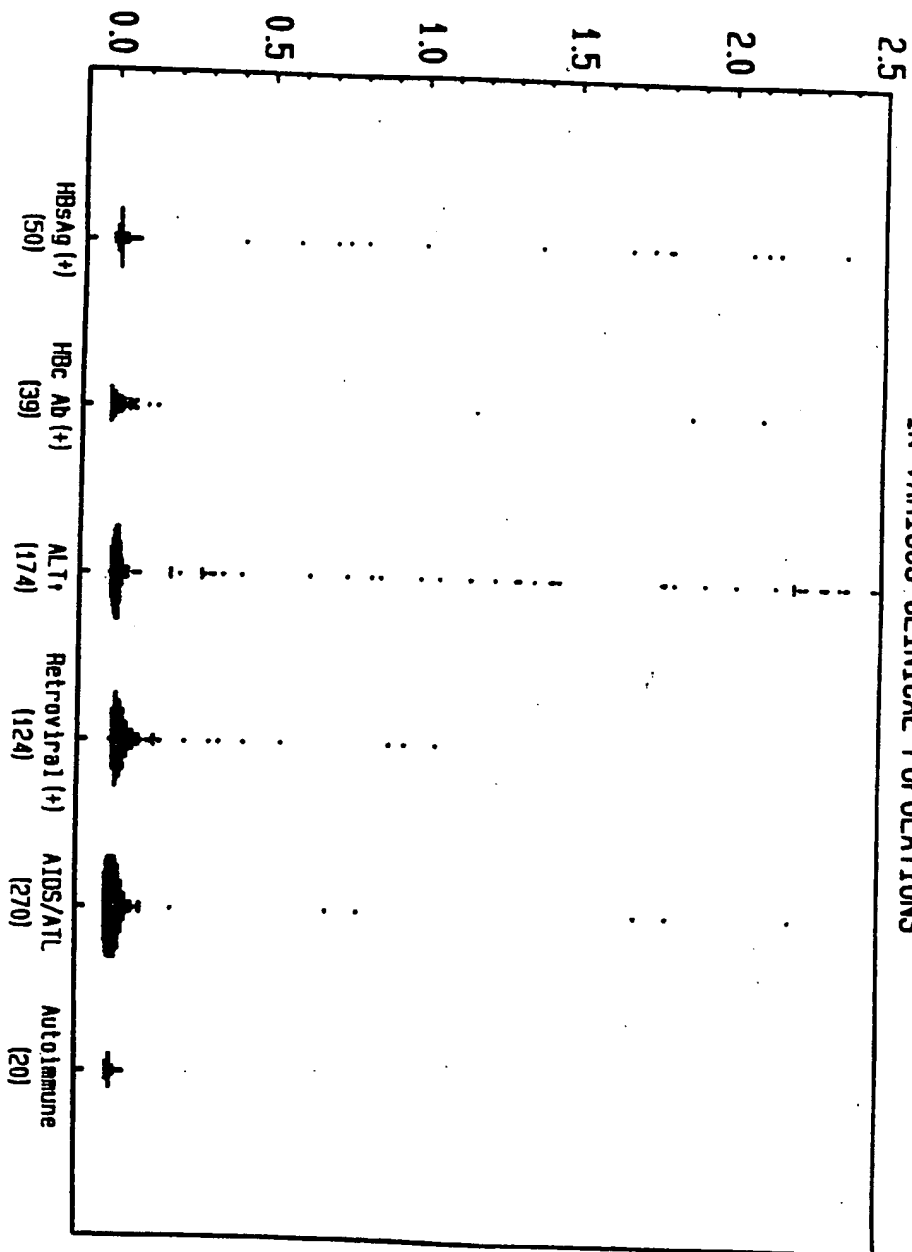
COMPARISON OF S/CUTOFF BETWEEN  
NORMAL PLASMA (n=264) AND NORMAL SERUM (n=264) SPECIMENS



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AS ORIGINAL FILED

07/558799

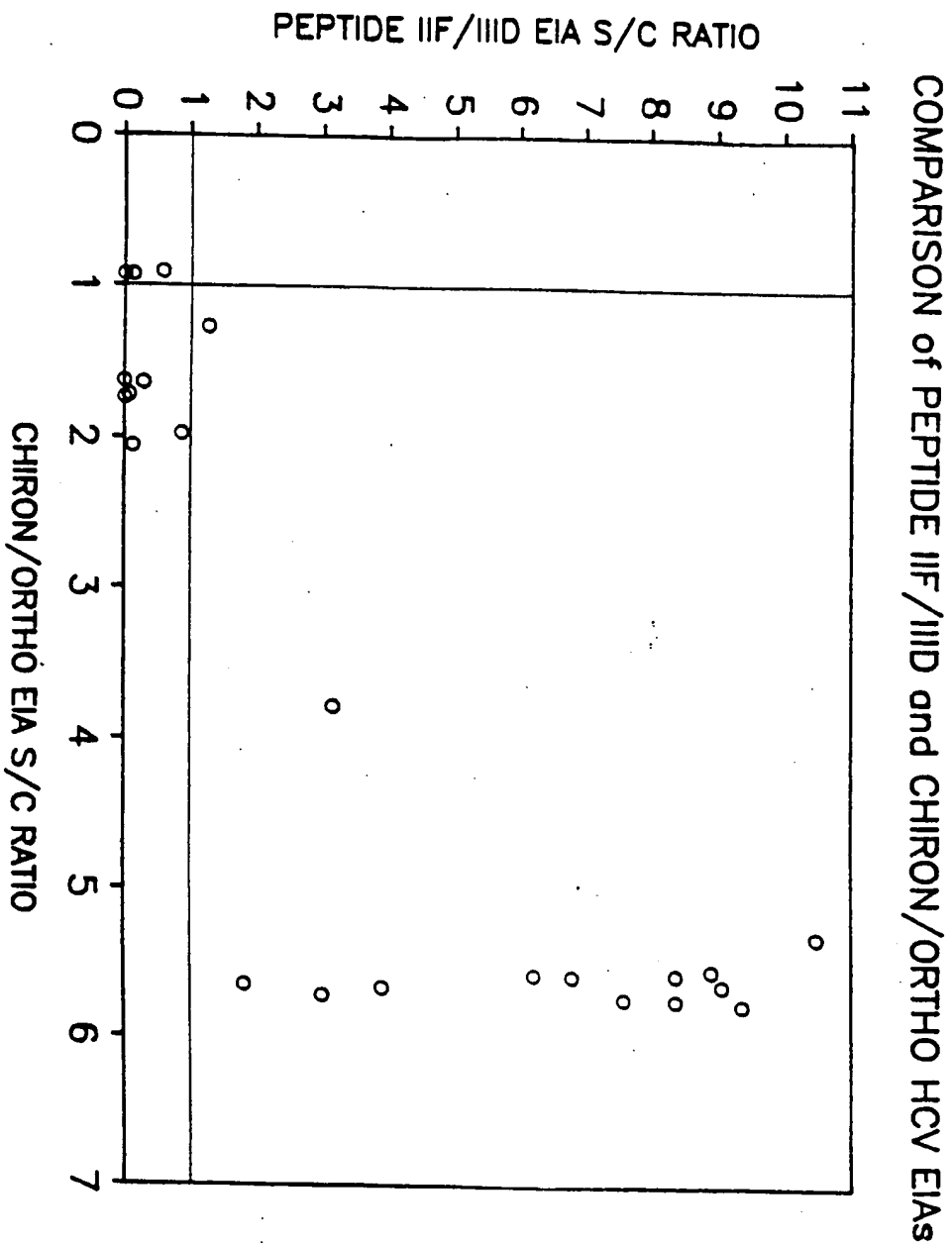
A492nm



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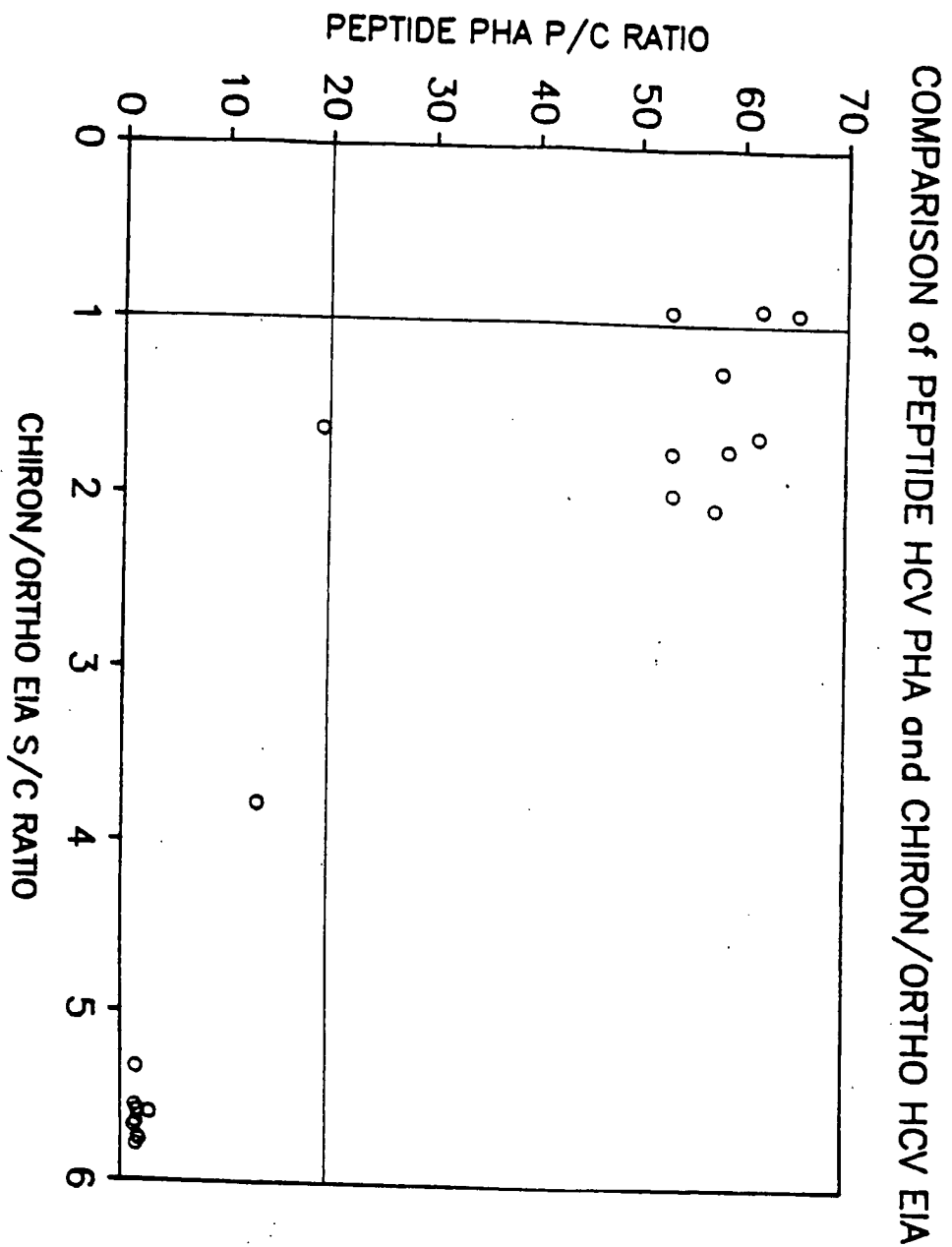
Fig 5



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*1.0 g/L*

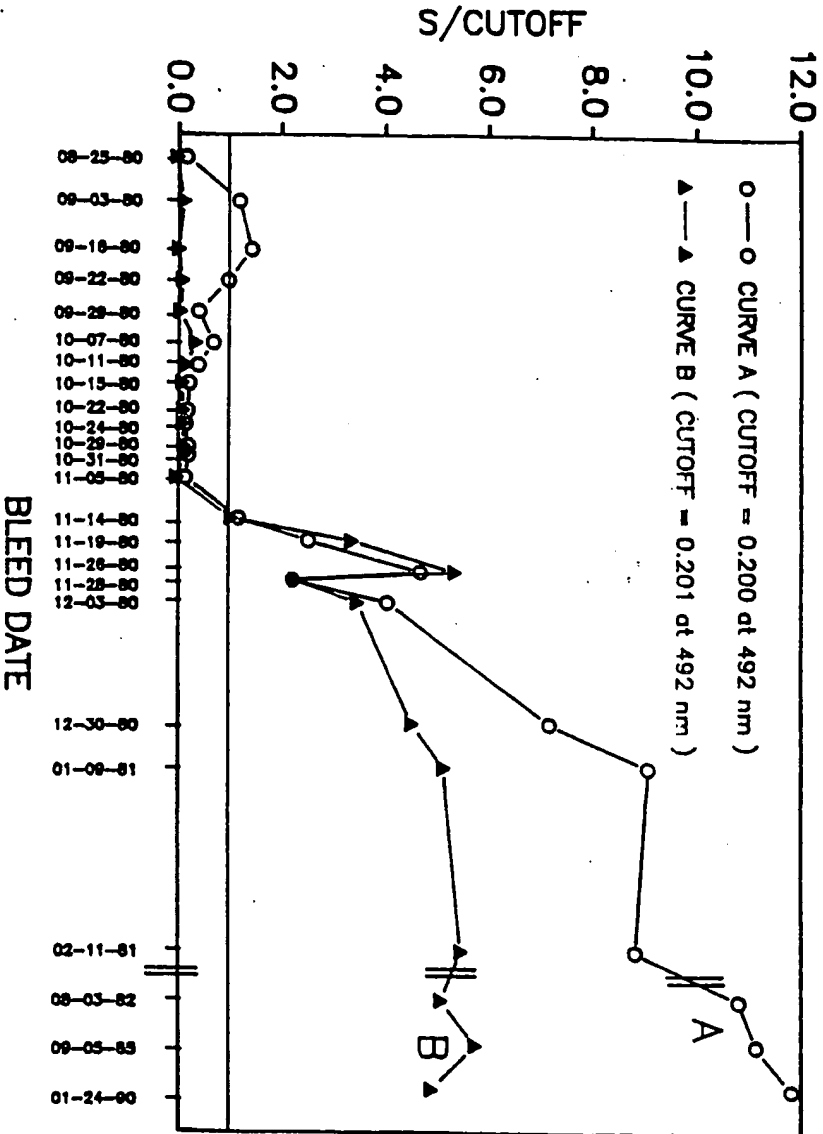




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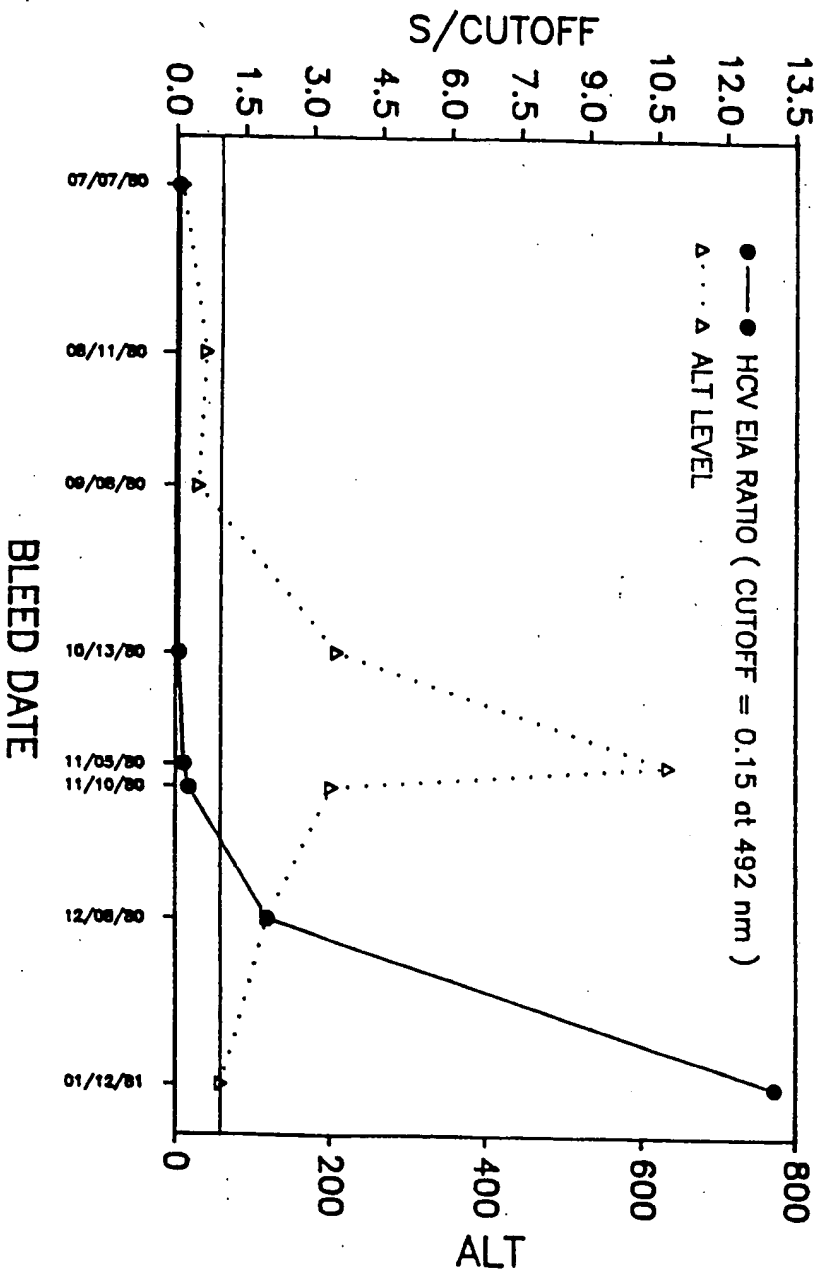
7-1  
N/A

# SEROCONVERSION PANEL I TRANSFUSION TRANSMITTED PATIENT (NIH)



N.Y.C. 7-2

SEROCONVERSION PANEL II  
DIALYSIS PATIENT WITH NANBH (NYBC)

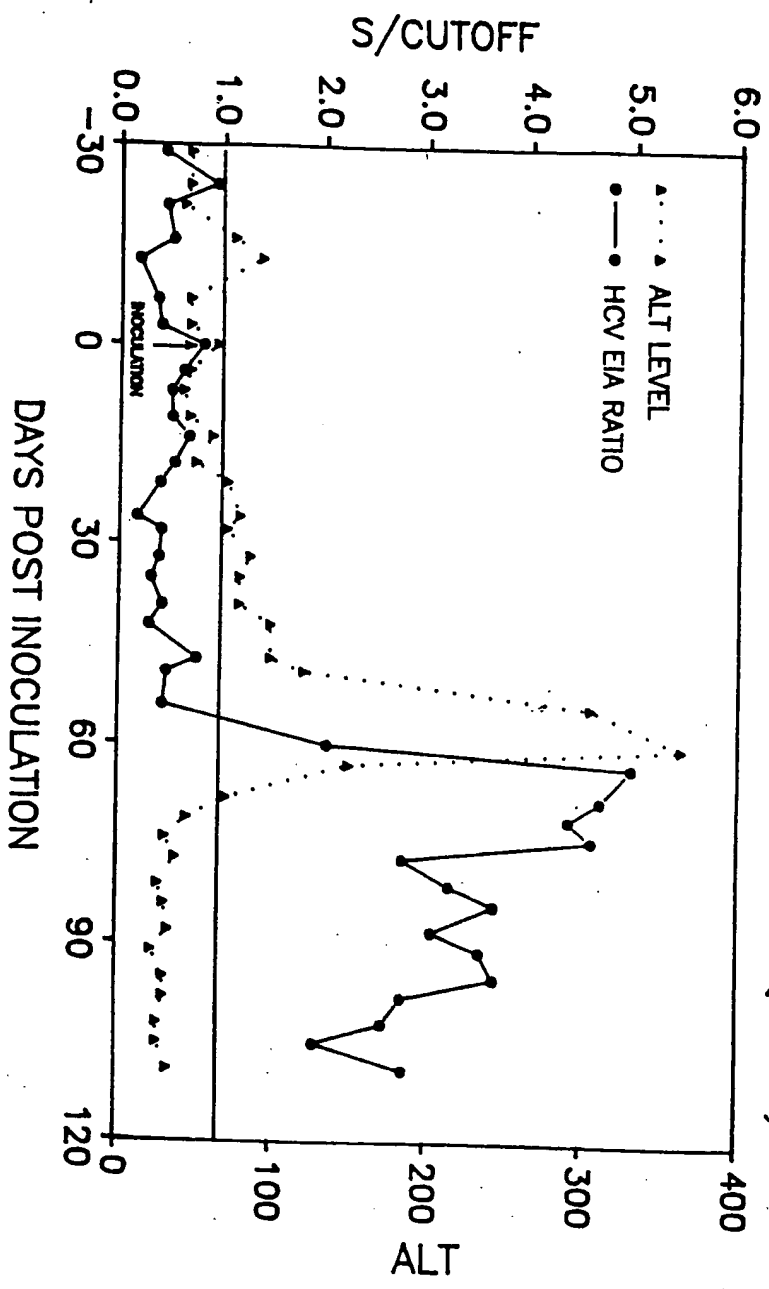


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*Fig 7-3*

# SEROCONVERSION PANEL III NANBHV INOCULATED CHIMP (CDC)



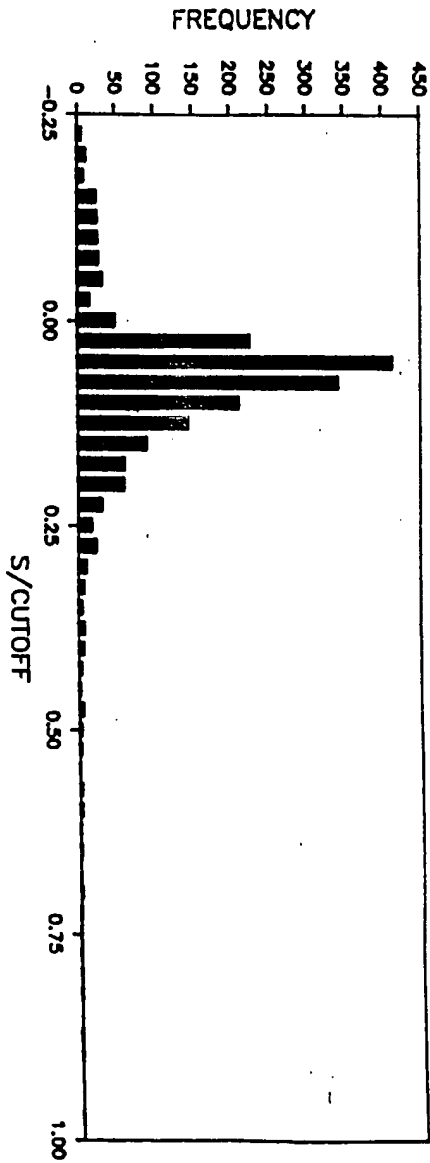
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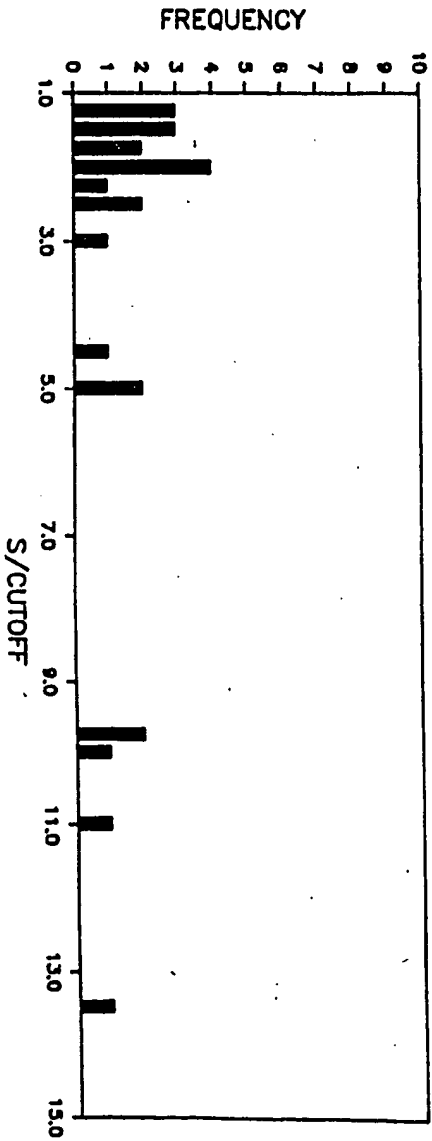
Fig 8-1

Fig 8-2

DISTRIBUTION of HCV-Ab NEGATIVE SPECIMENS ( n = 2011 )



DISTRIBUTION of HCV-Ab POSITIVE SPECIMENS ( n = 24 )

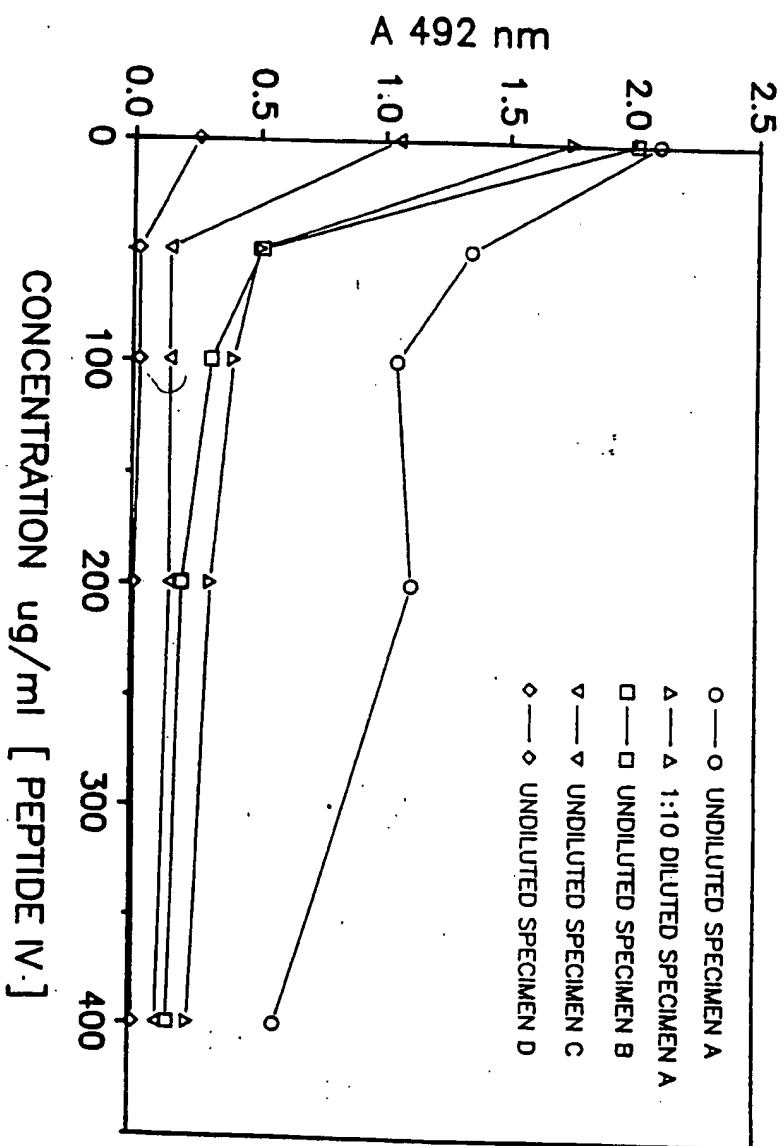


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10/8/81

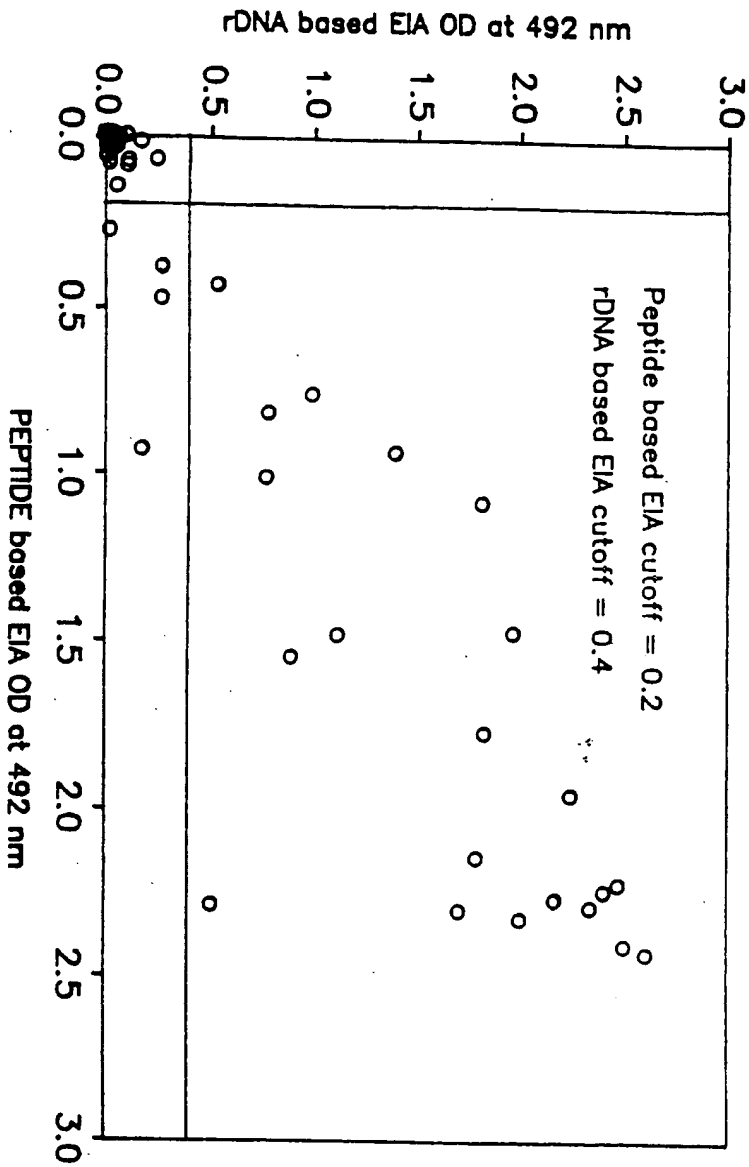
# SYNTHETIC PEPTIDE BASED HCV NEUTRALIZATION EIA AS A CONFIRMATORY TEST



07/558799

Fig 10

COMPARISON of PEPTIDE based and rDNA based HCV EIAs



077558799

EPITOPE MAPPING OF AN IMMUNODOMINANT REGION OF  
THE HCV CORE (NUCLEOCAPSID) PROTEIN (VIII A-E)

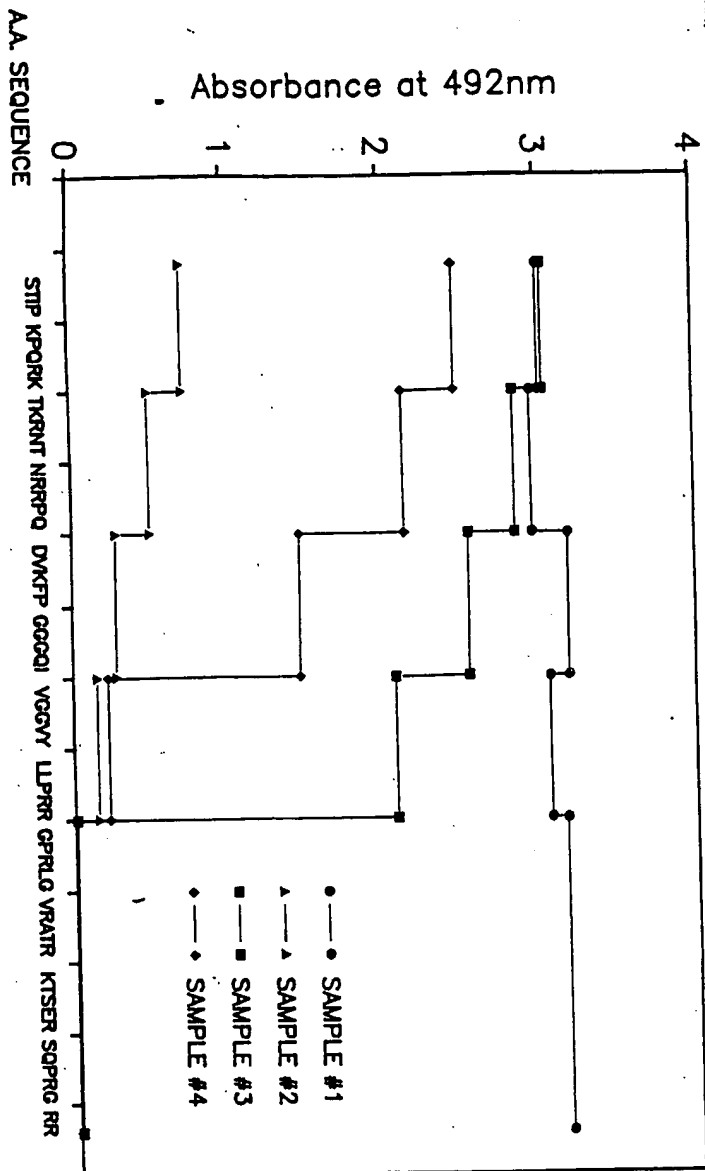


Fig 11-1

07/558799

# EPITOPE MAPPING OF AN IMMUNODOMINANT REGION OF THE HCV CORE (NUCLEOCAPSID) PROTEIN (IX A-E)

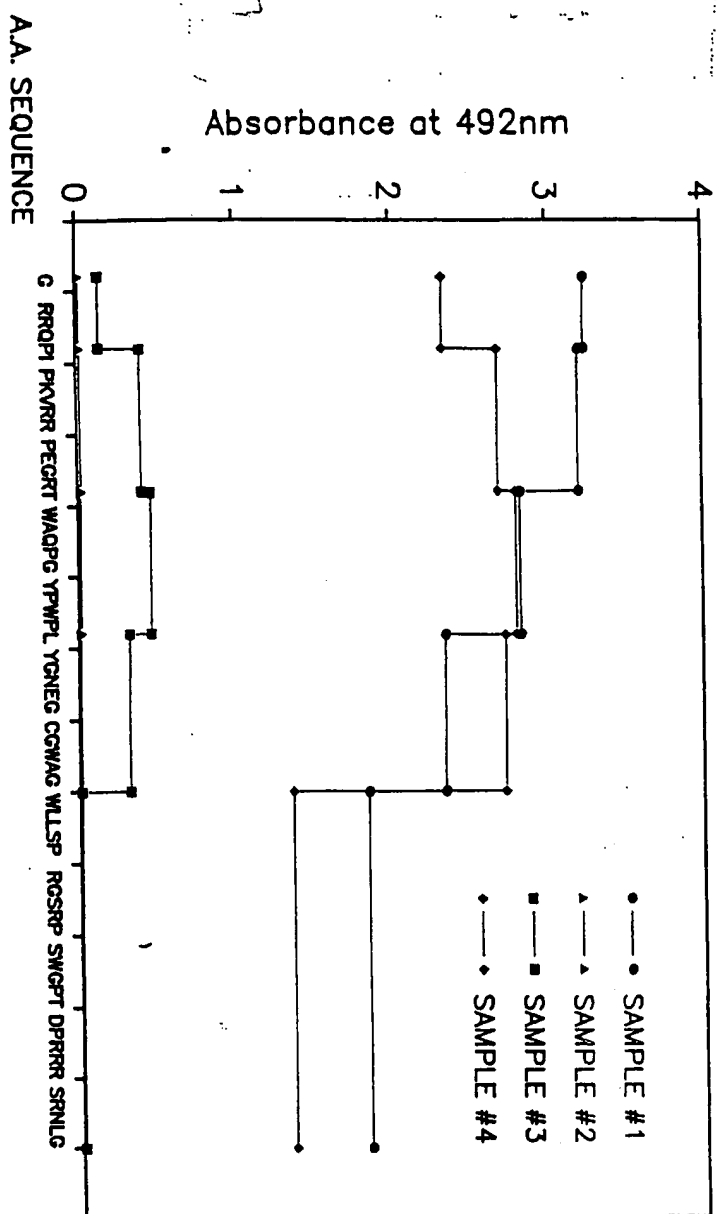


Fig 11-2



HCV POSITIVITY OF SPECIMENS BY FORMAT C

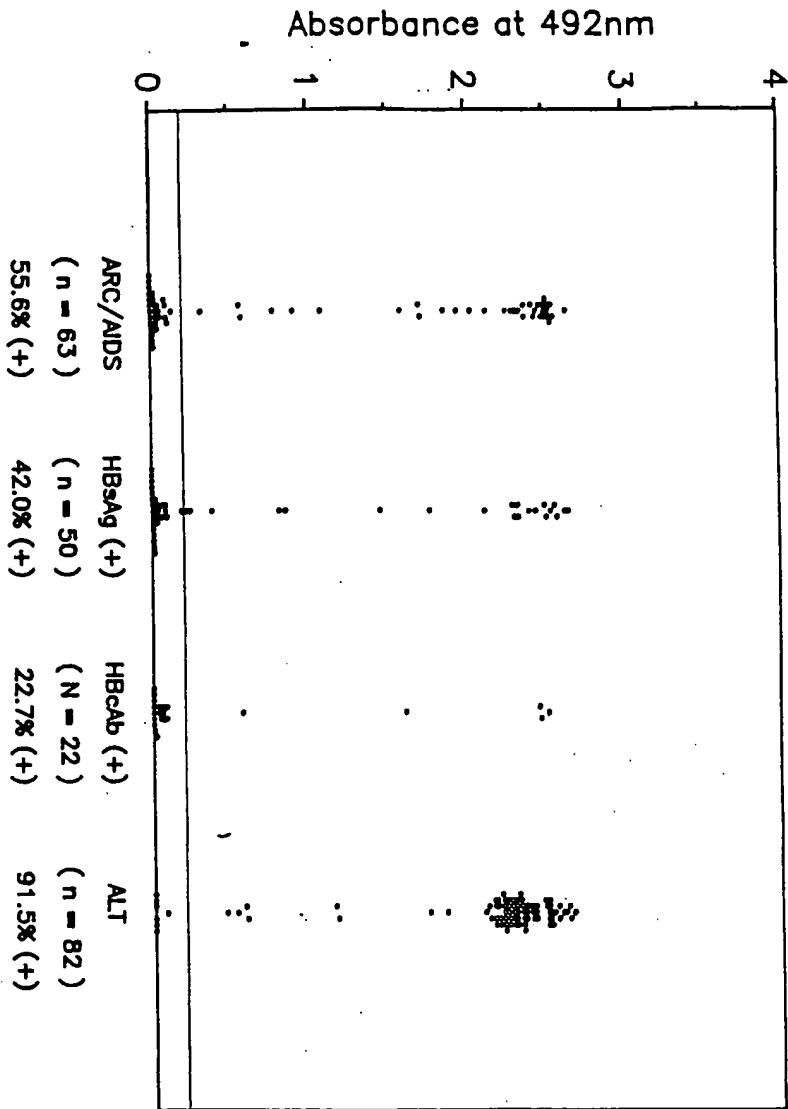


Fig 12-1

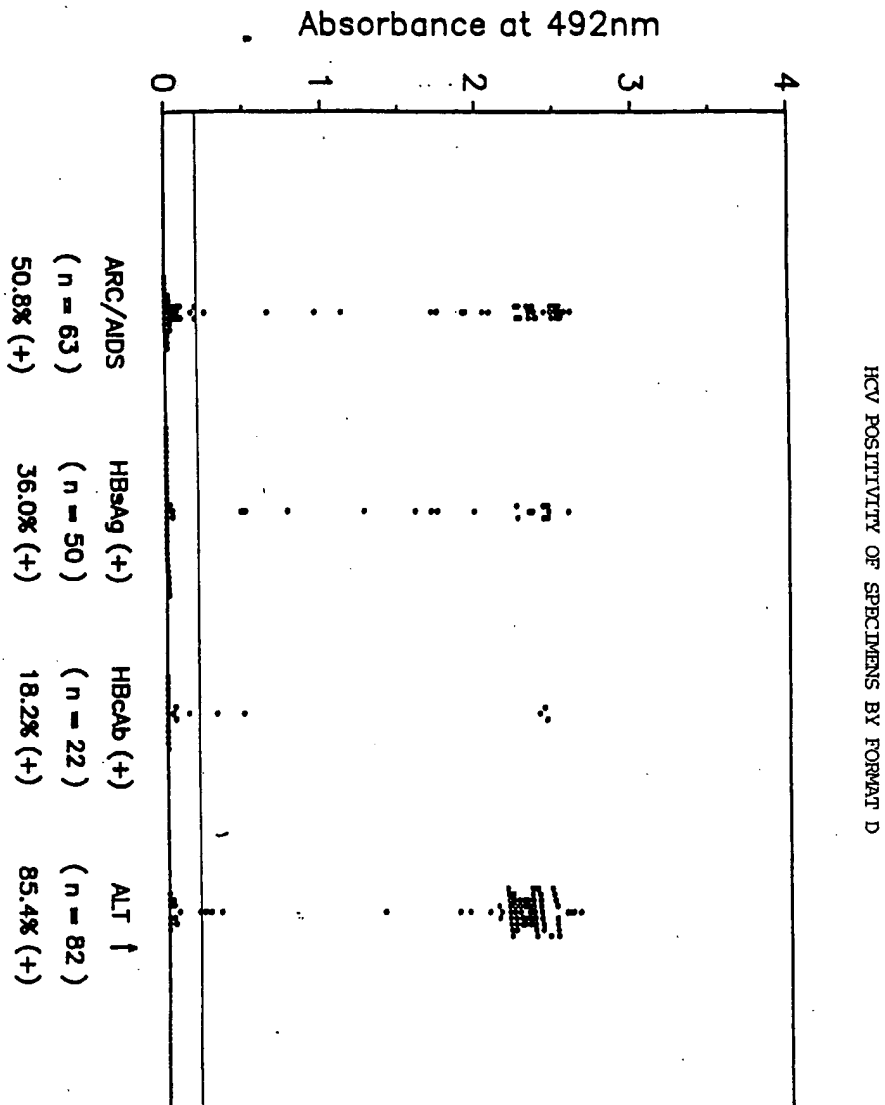
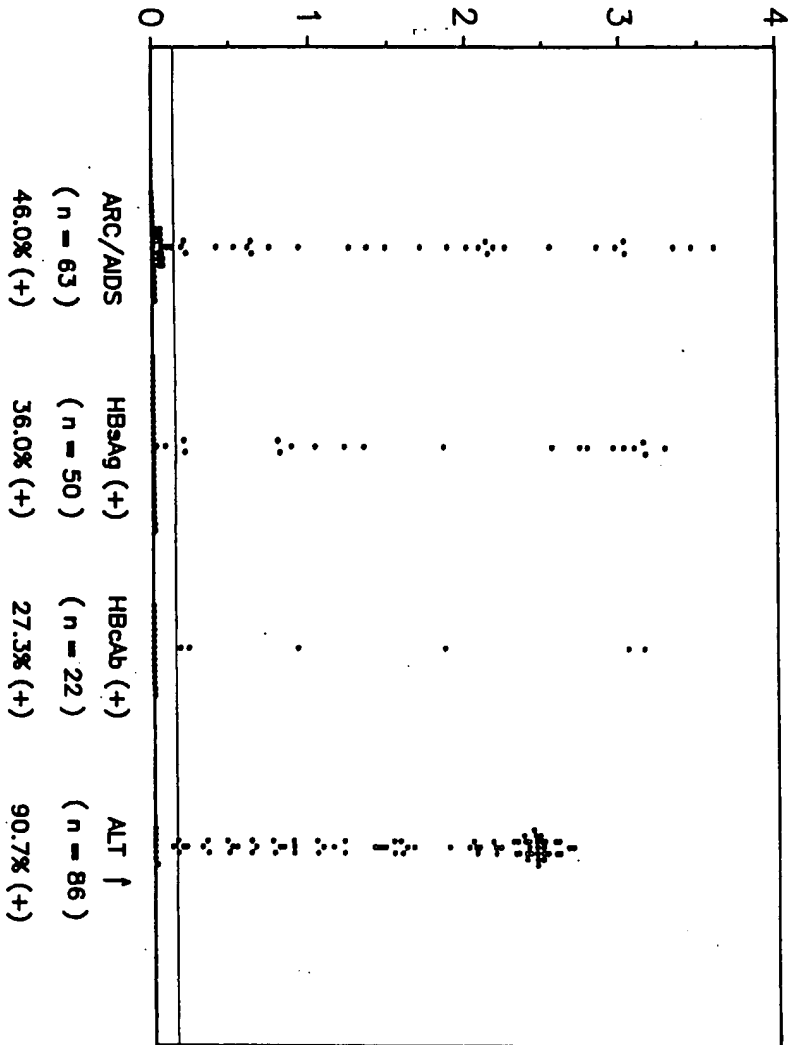


Fig 12-2

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07/558799

Absorbance at 492nm



HCV POSITIVITY OF SPECIMENS BY FORMAT A

Fig. 12-3

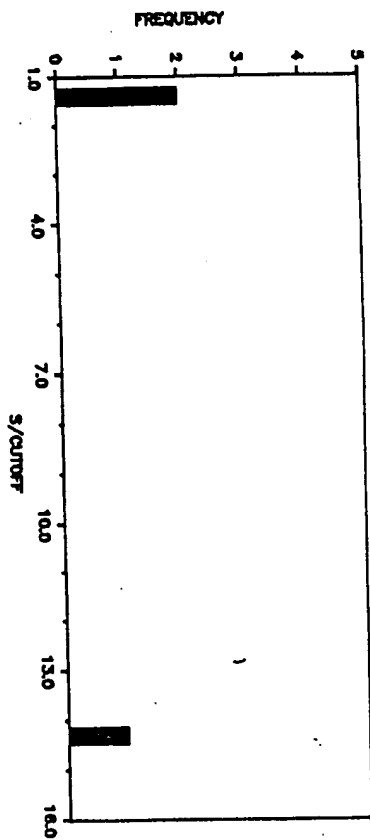


Fig 13-2

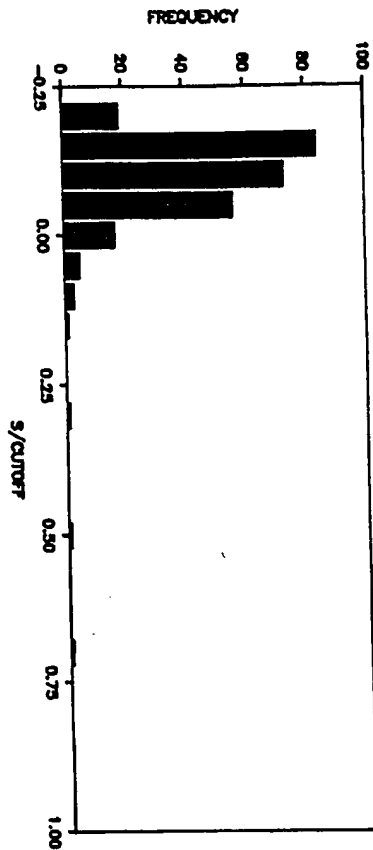


Fig 13-1

HCV POSITIVITY OF SPECIMENS (n=264) BY FORMAT A

07/558799

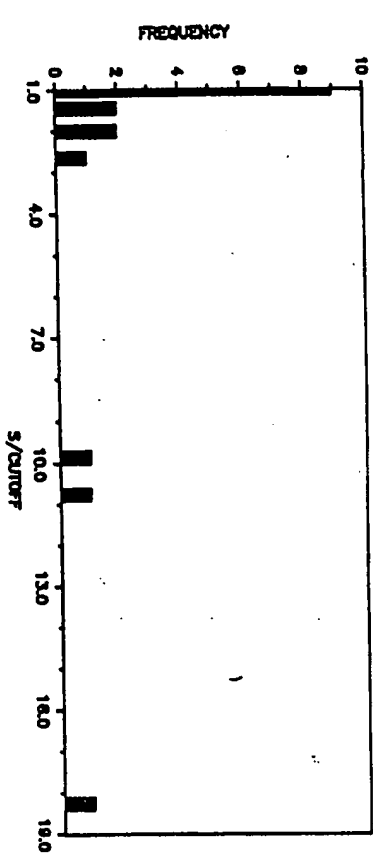


Fig 13-4

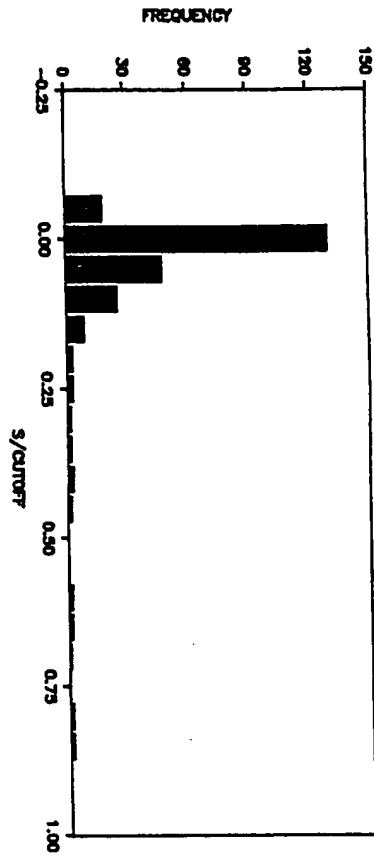


Fig 13-3

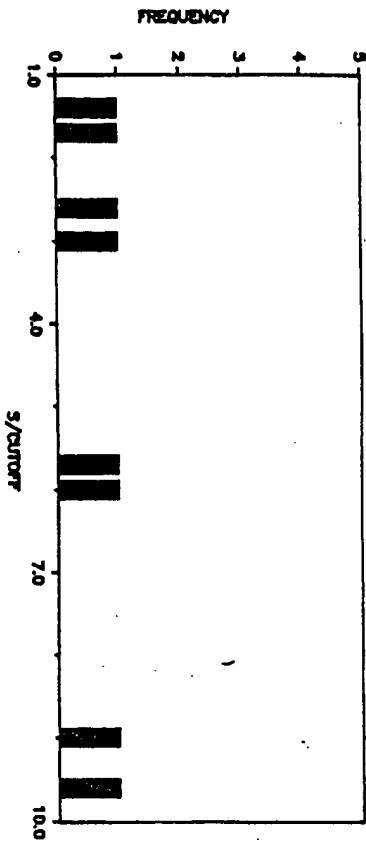


Fig 13-6

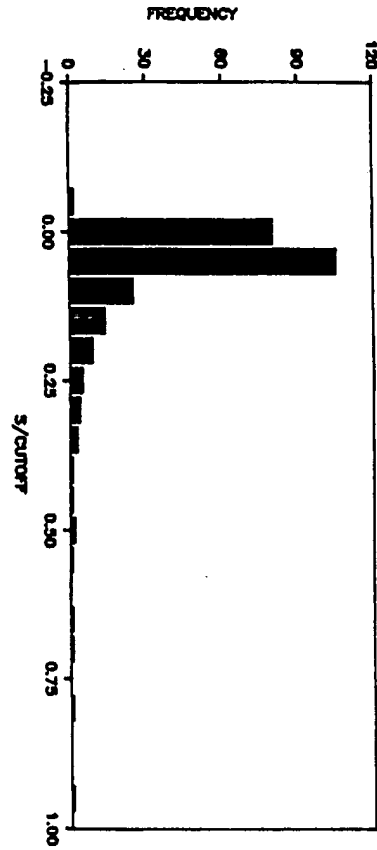


Fig 13-5

HCV POSITIVITY OF SPECIMENS (n=264) BY FORMAT D

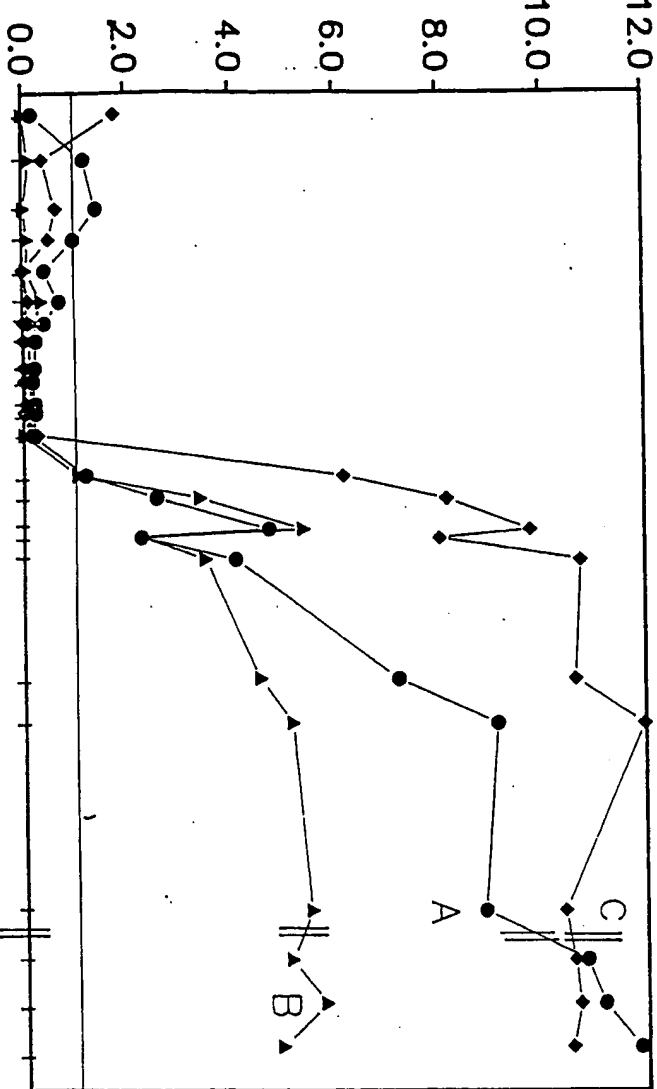
07/558799

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# SEROCONVERSION PANEL I TRANSFUSION TRANSMITTED PATIENT (NIH)

BLEED DATE

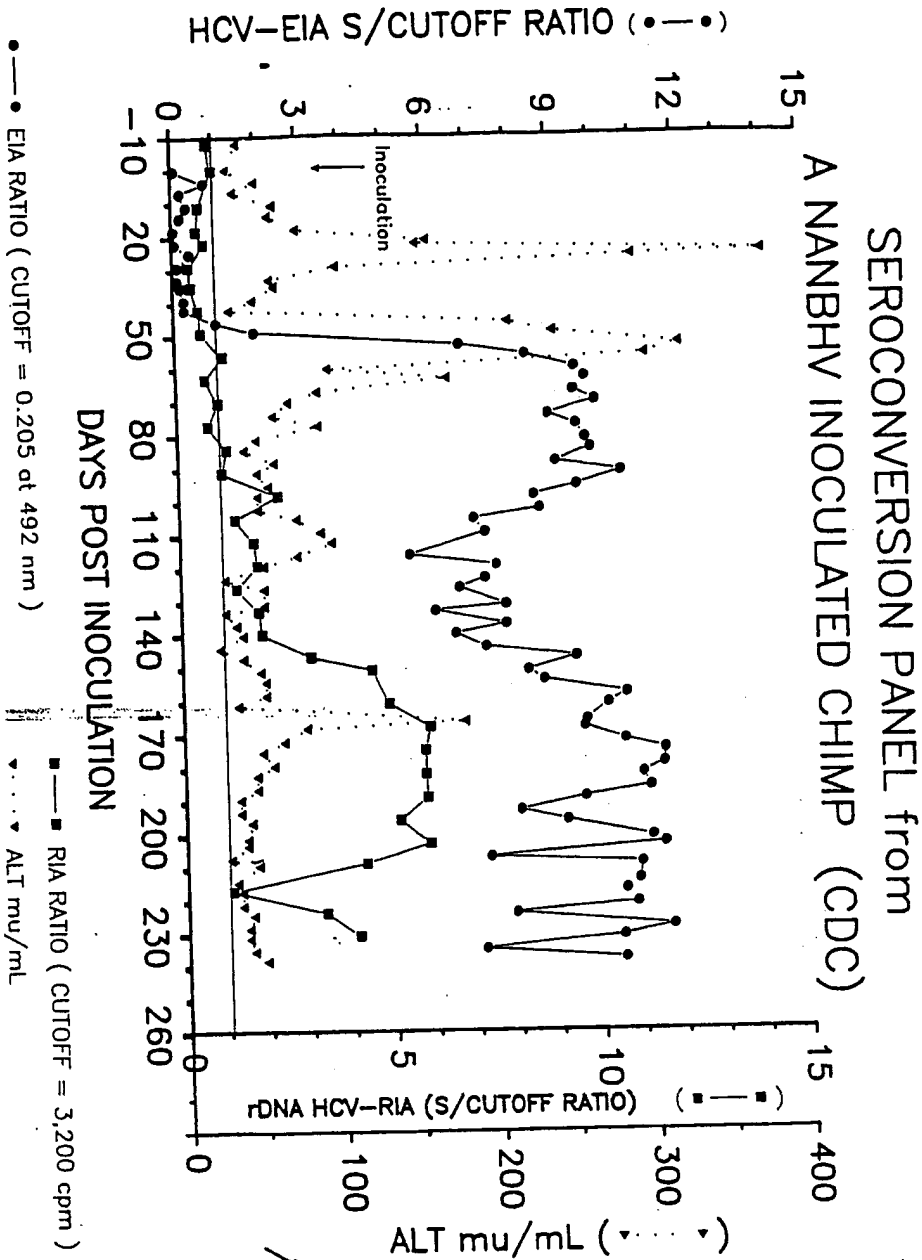
S/CUTOFF



- CURVE A ( CUTOFF = 0.200 at 492 nm )
- ▲ CURVE B ( CUTOFF = 0.201 at 492 nm )
- ◆ CURVE C ( CUTOFF = 0.205 at 492 nm )

Fig 14-1

07/558799





07/558799

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COMPARISON of PEPTIDE based (FORMAT C) and rDNA based HCV EIAs

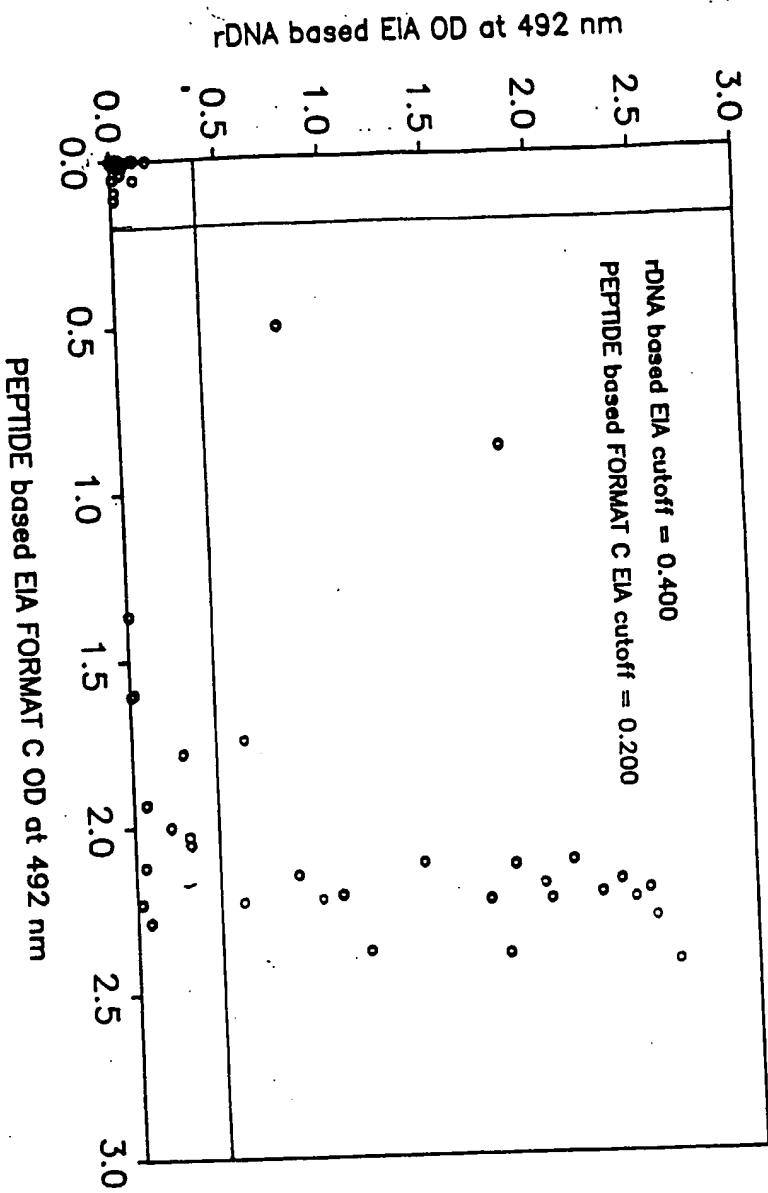


Fig 15-1

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07/558799

# COMPARISON of FORMAT C and FORMAT A PEPTIDE based EIAs

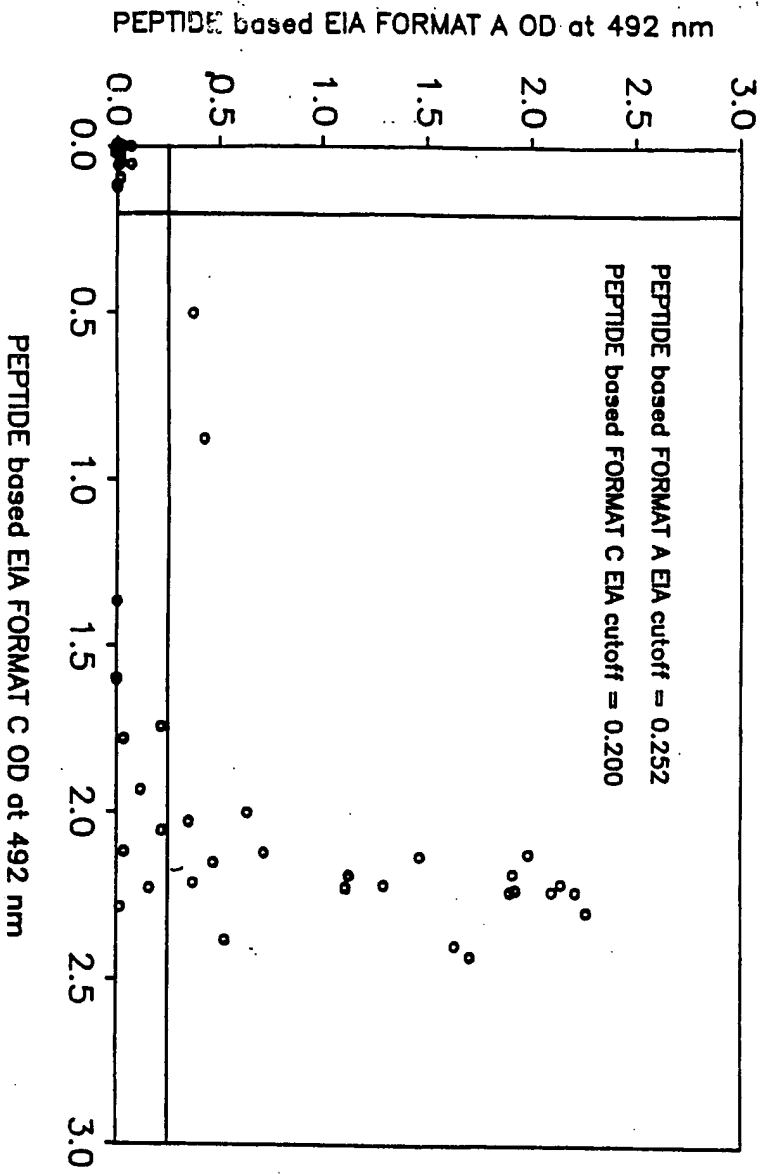


Fig 15-2

07/558799

PATENT

Docket No. 1151-4043

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

## APPLICATION FEE TRANSMITTAL

HON. COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

Sir:

Transmitted herewith for filing is the patent application of

Inventor(s): Chang Yi WangFor : SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV,: DIAGNOSIS OF HCV INFECTION AND PREVENTION THEREOF AS VACCINES

Enclosed are:

☒ 78 page(s) of specification☒ 1 page(s) of Abstract☒ 13 page(s) of claims☒ 27 sheets of drawing [ ] formal [x] informal☒ 6 sheets of Declaration and Power of Attorney☒ An assignment of the invention to United Biomedical Inc.☐ Claim to Convention Priority form and a certified copy of a \_\_\_\_\_ application.

## CALCULATION OF APPLICATION FEE

For	Number Filed	Number Extra	Rate	Basic Fee
Total	43	- 20 = 23		\$370.00
Claims*			x \$12.00	\$ 276.00
Independent				
Claims	5	- 3 = 2	x \$36.00	\$ 72.00
Multiple	[ ] yes	Add'l Fee \$120.00		
Dependent				
Claim(s)	<input checked="" type="checkbox"/> no	Add'l Fee NONE	= \$	

Total: \$618.00☒ Verified Statement of "Small Entity" Status Under 37 C.F.R. § 1.27Filed herewith.Reduced Fees Under 37 CFR § 1.9(f) (50% of total) paid herewith \$185.00

\* Includes all independent and single dependent claims and all claims referred to in multiple dependent claims. See 37 C.F.R. § 1.75(c)

PATENT

Docket No. 1151-4043

[ ] Charge fee to Deposit Account No. 13-4500. Order No. \_\_\_\_\_.  
A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

[x] The Commissioner is hereby authorized to charge any additional fees which may be required for filing this application, or credit any overpayment to Deposit Account No. 13-4500. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

☐ A check in the amount of \$ 185.00 to cover the filing fee is attached.

☐ A check in the amount of \$8.00 for recording the Assignment is attached.

Respectfully submitted,

MORGAN & FINNEGAN

Dated:

July 26, 1990

By:

Maria C.H. Lin  
601 Maria C.H. Lin  
Registration No. 29,323 301

Mailing Address:

MORGAN & FINNEGAN 602  
345 Park Avenue 701  
New York, New York 10154 702  
(212) 758-4800  
(212) 751-6849 Telecopier

07/25/90



PATENT

Docket No. 1151-4043

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Chang Yi Wang                      ~Group Art Unit: To be assigned  
Serial No. : To be assigned                      Examiner: To be assigned  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF  
ANTIBODIES TO HCV, DIAGNOSIS OF HCV INFECTION AND  
PREVENTION THEREOF AS VACCINES

EXPRESS MAIL CERTIFICATE

Express Mail Label No. LB211744522

Date of Deposit July 26, 1990

I hereby certify that the following attached paper(s) or fee  
Application Transmittal Form with check of \$185.00  
Specification 78 pages                      Assignment and Request for Recordation  
Claims 13 pages                              with check of \$8.00  
Abstract 1 page  
Informal drawings 27 sheets              Verified Statement of Small Entity  
Declaration and Power of Attorney

is being deposited with the United States Postal Service "Express Mail Post  
Office to Addressee" service under 37 C.F.R. §1.10 on the date indicated above  
and is addressed to the Commissioner of Patents and Trademarks, Washington, D.C.  
20231.

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12-14-90

1151-4043

PATENT  
U.S.S.N. 07/558,799

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Chang Yi Wang  
Serial No. : 07/558,799  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE  
DETECTION OF ANTIBODIES TO HCV, DIAGNOSIS  
OF HCV INFECTION AND PREVENTION THEREOF  
AS VACCINES  
Group Art Unit : 189/b  
Examiner : Lester Lee ✓

Hon. Commissioner of Patents and Trademarks  
Washington, D.C. 20231

INFORMATION DISCLOSURE STATEMENT

Sir:

Pursuant to 37 C.F.R. §§ 1.56 and 1.98, applicant, through her attorneys, makes of record the following document which the Examiner may consider to be relevant to the examination of the present invention as described and claimed. Copies of the listed documents in two binders with an index list of the references are attached herewith, together with a completed Form PTO - 1449.

THE CITED DOCUMENTS

It is respectfully requested that these documents be (1) fully considered by the Examiner during the course of examination of this application, (2) be listed in the "Notice of References Cited" issued in this application, and (3) printed on any application issuing from this application.

Applicant regrets that other office matters prevented this statement from being filed earlier.

Among the documents listed, References 1-29 were listed in the application. References 30-65 have come to the applicant's attention since the present application was filed.

Due to the volume of reference articles, a discussion was had with the Examiner, who has contacted the Applicant's attorney in connection with the requirement for restriction. It was agreed that only a summary of the group of references which provides a background history of the discovery of the disease and the etiological studies will be discussed in this statement.

The more pertinent references will be discussed individually. The applicant will try to point out which of the references are the most pertinent references to facilitate examination of the claims.

The present application is directed to specific synthetic peptides which have been found to be highly specific and effective in the diagnosis of HCV infection. Nine peptides, their analogues, combinations and mixtures have been described and claimed. Seven of the peptides embrace immunodominant epitopes from the putative non-structural protein of the HCV and two of the peptides embrace immunodominant epitopes from the putative structural protein of the HCV. The peptides have been found to provide the capability of detecting HCV infection about three months earlier than the only commercially available reagent, the HCV SOD-C100 fusion protein. Moreover, the detection rate is much higher showing that the peptides of the present application is much more sensitive and accurate.

The following is a discussion of the references.

Much of the literature is historical and describes the etiological studies of NANBH (1-15, 30-42), and the many attempts to isolate, purify and identify the

causative agent (16, 17, 18, 27, 29, 43, 45-48, 50). Only a summary of these references will be provided here.

The first recognition of the existence of a form of hepatitis associated with blood products and transfusion occurred in England in 1944(1). Based on clinical evidence using human subjects, two forms of hepatitis were observed, an infectious type (Hepatitis A) and a serum related type (Hepatitis B) (2, 3). Since the development of specific serological markers for HAV and HBV, it became increasingly obvious that another form of transfusion associated hepatitis exists. In 1974, Prince et al. first showed, by statistical analysis of available test data, the existence of a non-A-non-B viral type hepatitis, NANBH, caused by an unknown agent, which he named Hepatitis Virus type C (4). NANBH was confirmed by others by studying data from post-transfusion hepatitis patients who received blood units which were pre-screened to eliminate HBV and further analyzed to show the absence of HAV (5, 6, 7, 9, 33, 36, 38). The etiology of NANBH has been summarized in great detail (30, 42). In a slide presentation to the American Association of Blood Banks (30), Abbott described the various types of hepatitis in a brief and succinct manner. The transfusion associated NANBH agent is now called the Hepatitis C Virus (HCV). It describes a specific marker for HCV which has been developed by Chiron Corporation. This specific marker is a fusion protein of human superoxide dismutase (SOD) with an HCV antigen described as a composite of clones 81, 32 and 36 also called HCV C 100-3, corresponding to a non-structural gene of the HCV. This marker, when used in a radioimmunoassay detects HCV antibodies in 15-25% of the clinically diagnosed acute cases of NANBH, 67-85% of the clinically diagnosed cases of chronic NANBH. Between 0.2 and 1.2% of random donors were found reactive for HCV antibody. Because the marker failed to detect 100% of NANBH,



this reference suggested that a different viral antigen (preferably structural) in an EIA, western blot or other formats may be useful.

There have been many attempts to isolate and identify the causative agent for NANBH, however, until recently the lack of success has prompted several authors to suggest the use of surrogate tests, such as elevated liver enzyme activity (ALT) as an indicator to screen the blood supply to avoid the transmission of NANBH. One difficulty with ALT as a surrogate marker is where to set the lower limit for the exclusion of donors without affecting the amount of blood available for transfusion. It has been shown that even by setting a low level,  $\geq 45$  IU/L, only 29% of the cases of NANBH would have been prevented (10). Others had suggested the use of both anti-HBc positivity and elevated ALT as surrogate indicators for the screening of donor blood samples (11, 12, 14, 15, 32, 38, 40, 41). Statistical analysis show that this would also have only prevented 30% of transfusion associated NANBH. At the same time, about 8-9% of the donors would be excluded, further reducing the available blood supply. These difficulties of using the surrogate tests were further discussed by Polesky et al. (39).

Moreover, there is a need for finding a specific marker for HCV for diagnostic purposes. Present testing calls for a panoply of tests including liver enzyme activity, bilirubin, HBsAg, anti-HBs, anti-HBc, and absence of EBV (Epstein-Barr Virus), and CMV (Cytomegalovirus). This panoply of tests is very costly, and the results are uncertain.

References 19-26 describes immunodominant epitopes for HIV virus. This shows that synthetic peptides have been found to be useful for diagnosis of another diseases. However, these references do not relate at all to HCV and are, therefore, not pertinent.

Many attempts have been made to obtain HCV in an isolated and purified form. These references will be discussed individually hereinbelow.

16 & 17. Choo et al., Science, 244:359-362 (21 April 1989), and Kuo G. et al. Science, 244:362 (1989) describe the isolation of a cDNA clone derived from a genome library constructed from pooled serum from a chimpanzee known to contain NANBH viral material. The library was screened for rare clones expressing viral antigen with the serum of a patient with chronic NANBH. Using this technique, two positive clones were identified, clone 5-1-1 and clone 81. Clone 5-1-1 was then recombinantly expressed as a fusion polypeptide with human superoxide dismutase (SOD) (16). The SOD/HCV clone 5-1-1 was found to be useful as a specific marker for HCV for the diagnosis of NANBH (17).

18. EP 0318216A1 (17) (Chiron application) describes in detail the isolation of a family of cDNA clones of portions of the HCV genome from a chimpanzee. Among the 26 clones so isolated were clones 5-1-1 and 81 reported in the Choo et al. and Kuo et al. articles (16, 17). The amino acid sequence of clone 5-1-1, 51 amino acids, was given in Figure 1:

Ala-Ser-Cys-Leu-Asn-Cys-Ser-Ala-Ser-Ile-Ile-Pro-  
Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-  
Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-  
Leu-Gly-Leu

The amino acid sequence for clones 5-1-1, 81, 91 & 1-2 was given in Fig. 3:

Gly-Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-  
Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-  
Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-  
His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-

Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-  
 Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-  
 Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-  
 Ala-Lys-His-Met-Trp-Asn-Phe-Ile-Ser-Gly-Ile-Gln-  
 Tyr-Leu-Ala-Gly-Leu-Ser-Thr-Leu-Pro-Gly-Asn-Pro-  
 Ala-Ile-Ala-Ser-Leu-Met-Ala-Phe-Thr-Ala-Ala-Val-  
 Thr-Ser-Pro-Leu-Thr-Thr-Ser-Gln

The amino acid sequences for the remaining clones are given in Figs. 4-32. Among the remaining clones, a fusion protein of SOD with a composite DNA of clones 81, 36 and 32 was also described. It was designated as HCV C-100-3. This is the antigen used in the commercially available test kit produced and sold by Chiron and Ortho as SOD/HCV C-100. The application is seventy three pages long with sixty three pages of drawings. Therefore, the above is only a summary of its most pertinent parts.

27. Okamoto et al. Japan. J. Exp. Med., 60:167-177 (1990)

describe obtaining other cDNAs from a Japanese chronic NANBH patient and a chimpanzee. Both samples were confirmed for infectivity for NANBH by chimpanzee transmission experiments. Using the cDNA obtained, the 5'-terminal sequence of the genome of HCV was determined for both strains (27). Okamoto et al. postulated that this segment contains the structural genes for HCV and predicted the amino acid sequence of the product of the cDNA as follows:

(a)	MSTIPKPQRKTKRNTNRRPQDVKFPGGGQIVGGVYLLPRRGPRLGVRATR	50
(b)	---N-----	
	KTSESRQPRGRRQPIPKVRRPEGRTWAQPGYPWPLYGNEGCGWAGWLLSP	100
	-----W-----A-----A-----L-----	
	RGSRPSWGPTDPRRRSRNLGKVIDTLTCGFADLMGYIPLVGAPLGGAARA	150
	-----	
	LAHGVRVLEDGVNYATGNLPGCSFSIFLLALLSCLTVPASAYQVRNSTGL	200
	-----I-----E---VS-I	

	YHVTNDCPNSSIVYEAHDAILHTPGCVPCVREGNVSRCWVAMTPTVATRD	250
	-----S-----A-M-M-----D-S-----L---L-A-N	
	GKLPATQLRRHIDLLVGSATLCSALYVGDLGCVFLIGQLFTFSPPRRHWT	300
	ASV-T-TI---V-----A-AF---M-----VS-----E-	
	TQGCNCSIYPGHITGHRMAWDMMNWSPTAALVMAQLLRIPQAILDMIAG	350
	V-D-----LS-----T---VS-----VV--V--	
	AHWGVLAGIAYFSMVGWAKVLVLLLFAGVDAETIVSGGQAARAMSGLV	400
	-----L--Y-----I-A-----YT---A-SHTT-T-A	
	SLFTPGAKQNIQLINTNGSWHINSTALNCNESLNTGWLGLIYQHKFNSS	450
	---S---S-R---V-----R-----D--H--F--A-F-T-R---	
(a)	GCPERLASCRRLTDFDQGWGPISHANGSGPDQRPYCWHPKPCGIVPAK	500
(b)	-----M-----IDW-A-----TYTEPDS-----A-R-----S	
(c)	-----Y-----	
	SVCGPVYCFTPSP	550
	Q-----	
	-----VVVGTTDRSGAPTYSWGENDTDVFLNNTRPPLGNWF	
	GCTWMNSTGFTKVCGAPPCVIGGAGNNTLHCPTDCFRKHPDATYSRCGSG	600
	PWITPRCLVDYPYRLWHWPCTINYTIFKIRMYVGGVEHRLEAACNWTRGE	650
	RCDLEDRDRSELSPLLLTTTQWQVLPSCFTTLPALSTGLIHLHQNIVDVQ	700
	YLYGVGSSIASWAIKWEYVLLFLLLLADARVCSCLWMMLLISQAEALQN	750
	LVILNAASLAGTHGLVSFLVFFCFAWYLGKQWPGAVYTFYGMWPLLLLL	800
	LALPQRAYALDTEVAASCGGVVLVGLMALTLSPPYKRYISWCLWWLQYFL	850
	TRVQAQLHVWIPPLNVRGGRDAVILLMLAVHPTLVFDITKLLAVFGPLW	900
	ILQASLLKVPWFVRVQGLLRFCALARKMIGGHYVQMVIIKLGALTGTYYV	950
	NHLTPLRDWAHNGRLDLAVAVEPVVFSQMETKLITWGADTAACGDIINGL	1000
	PVSARRGREILLGPADGMVSKGWRLAPITAYAQQTRGLLGCIITSITGR	1050
	DKNQVEGEVQIVSTAAQTFLATCINGVCWTVYHGAGTRTIASPKGPVIQM	1100
	YTNVDQDLVGWPAPQGSRLTPCTCGSSDLYLVTRHADVIPVRRRGASRG	1150
	SLLSPROISYLGSSGGPLPCPAGHAVGIFRAAVCTRGVAKAVDFIPVEN	1200
	LETTMRSPVFTDNSSPPVPQSFQVAHLHAPTSGSKSTKVPAAYAAQGYK	1250
	VLVLNPSVAATLGFGAYMSKAHGIDPNIRTGVRTITTGSPITYSTYKFL	1300
	ADGGCSGGAYDIIICDELHSTDATSILGIGTVLDQAETAGARLVVLATAT	1350
	PPGSVTVPHPNIEEVALSTTGEIPFYGKAIPLEVIKGRHLIFCHSKKKC	1400
(a)		- 1450

(c) DELAAKLVALGINAVAYYRGLDVSVIPTSGDVVVVATDALMTGYTGDFDS

-----T-Y-----RR----- 1500  
VIDCNTCVTQTVDPSLDPTFTIETITLPQDAVSRTQRRGRTGRGKPGIYR

--T-----A-----S-----L----- 1550  
FVAPGERPSGMFDSSVLCECYDAGCAWYELTPAETTVRLRAYMNTPLPV

-----S-----A-D-F-----K-- 1600  
CNDHLEFWEGVFTGLTHIDAHFLSQTKQSGENLPYLVAYQATVCARAQAP

-----V-----I-- 1650  
PPSWDQMWKCLIRLKP TLHGPTPLLYRLGAVQNEITLTHPVTKYIMTCMS

ADLEVVTSTWVLVGGVLAALAAAYCLSTGCVVIVGRVVLSGKPAIIPDREV 1700

LYREFDEMEEC SQHLPYIENGMMLAENFKQKALGLLQTASRQAEVIAPAV 1750

QTNWQKLETFWAKHMWNFISGIQYLAGLSTLPGNPAIASLMAFTAAVTSP 1800

LTTSQTLLFNILGGWVAAQLAAPGAATAFVGAGLAGAAIGSVGLGKVLID 1850

ILAGWGAGVAGALVAFKIMSGEVPSTEDLVNLLPAILSPGALVVGVCVAA 1900

ILRRHVGPGEAVNWMNRLIAFASRGNHVSPTHYVPESDAAARVTAILSS 1950

LTVTQLLRRLHQWISSECTTPCSGSWLRDIWDWICEVLSDFKTWLKAKLM 2000

PQLPGIPFVSCQRGYKGVWRVDGIMHTRCHCGAEITGHVKNGTMRIVGPR 2050

TCRNMWSGTFPINAYTTGPCTPLPAPNYTFALWRVSAEEYVEIRQVGDFH 2100

YVTGMTTDNLKCPQVPSPEFFTEL DGVRLHRFAPPCKPLLREEVSFRVG 2150

LHEYFVGSQLPCEPEPDVAVLTSMLTDP SHITAEAGRRRLARGSPPSVAS 2200

SSASQLSAPSLKATCTANHDS PDALIEANLLWRQEMGGNITRVESENKV 2250

VILDSFDPLVAEEDEREISVPAEILRKSRRFAQALPVWARPDYNPPLVET 2300

WKKPDYEPVVHGCPLPPPKSPPVPPPRKKRTVVLTSTLSTALAEATR 2350

SFGSSSTSGITGDNNTTSSEPAPSGCPPDS DAESYSSMPPLEGEPGDPDL 2400

SDGSWSTVSSEANAEDVCCSMSYSWTGALVTPCAAEEQKLPINALSNSL 2450

LRHHNLVYSTTSRSACQRQKKVTFDRLQVLD SHYQDVLKEVAAAASKVKA 2500

NLLSVEEACSLTPPHSAKSKFGYGA KDVRCHARKAVTHINSVWKDLLEDN 2550

VTPIDTTIMAKNEVFCVQPEKGRKPARLIVFD LGVRVCEKMALYDVVT 2600

KLPLAVMGSSYGQYSPGQ RVEFLVQAWKSKKTPMGFSYDTRCFDSTVTE 2650

SDIRTEEA IYQCCDLDPQARVAIKSLTERLYVGGPLTNSRGENGCGYRRCR 2700

ASRASGVLTTSCGNTLT CYIKARAACRAAGLQDCTMLVCGDDL VVICESA 2750

GVQEDAASLRAFTEAMTRY SAPPGDPPQPEYDLELITSCSSNVSVAH DGA 2800

GKRVYYLTRDPTTPLARAAWETARHTPVNSWLGNIIMFAPTLWARMILMY 2850  
HFFSVLIARDQLEQALDCEIYGACYSIEPLDLPPIIQL 2889

29. Kubo et al. Nucleic Acids Research, 17:10367-10372

(November 24, 1989) describe obtaining cDNA from the plasma of a Japanese blood donor implicated in post-transfusion NANBH. The sequence showed 79.8% homology at the nucleotide level and 92.2% homology at the amino acid level compared with the prototype HCV cDNA obtained by Chiron from a chimpanzee in the United States (29). See Chiron application above.

43. Seto et al., U.S. Patent 4,673,634 (43), describes the isolation and purification of an antigen which is claimed to be specific for NANBH. The antigen was obtained from the serum of a patient who was diagnosed to have chronic NANBH. It was isolated by DEAE-cellulose chromatography, Con A-Sepharose affinity chromatography and DEAE-cellulose rechromatography. The antigen was tested by counterelectrophoresis using an antiserum from a chimpanzee which was inoculated with serum from a NANBH patient. Seto et al states that the isolated antigen is on the surface of the virus particle at a density of 1.14 g/ml and in soluble form having the following properties:

- a. Molecular weight of the glycoprotein monomer on sodium dodecyl sulfate polyacrylamide gel being about 77,000;
- b. reactive with the rhesus monkey immune serum;
- c. being a glycoprotein with about 2% carbohydrates, which are predominantly mannose, and about equal ratio of fucose and galactose;
- d. having an aromatic amino acid composition of phenylalanine, tyrosine, and tryptophan in a molecular ratio of 3:3:1; and
- e. being an immunogen.

Seto et al. showed the specificity of the isolated agent by testing it in a solid-phase radioimmunoassay using serum from patients diagnosed with chronic hepatitis, acute hepatitis, hepatitis A, hepatitis B and control samples. By using the agent, all 15 chronic hepatitis serum samples, none of the hepatitis A or Hepatitis B serum samples, and 3 out of 28 acute hepatitis serum samples were found to be positive. The amino acid content of the agent was determined, however, no specific amino acid sequence was disclosed.

44. Fount et al., U.S. 4,777,245 describe the development of a non-human primate monoclonal antibody specific against an antigen associated with NANBH infection.

Lymphocytes isolated from blood taken from an NANBH infected chimpanzee. The T cells were removed. The remaining B cells were transformed by EBV. The transformed cell were cultured and the cell supernatants assayed for NANB-specific antibodies by indirect immuno-fluorescence on NANB-infected liver sections. The positive cells were expanded and fused to form hybridomas and grown. Using ELISA techniques, the antibodies secreted were tested for specificity with NANB.

No NANB antigen or virus was described disclosed.

45 & 46. Tabor et al., U.S. Patent 4,356,164 and 4,395,395 (45, 46), describes a method of using antigen-antibody reaction in counterelectrophoresis to detect the presence of NANBH antigen. The anti-NANBH was isolated and purified from NANBH serum from a chimpanzee by precipitation with 30% ammonium sulfate. The anti-NANB was radiolabelled and tested using a known antigen-positive serum. Tabor et al. also described the use of several techniques which may be available for purifying the NANBH associated antigen.

47. Coursaget et al., U.S. Patent 4,464,474 (47), describe a virus particle isolated from the urine and serum of NANBH patients. The virus particle resembles a togavirus and is 50-60 nm in diameter with a discrete core of about 40 nm in diameter. These particles were found in sera of NANBH infected patients, but not in patients with acute NANBH. The particles were discovered by electron microscopy and replicated in vivo by culturing in primate diploid cell lines, such as MRC-5 and WI-38 lines. The viral particles were then separated from the cell debris by low speed centrifugation or filtration supplemented by sedimentation with a sucrose density gradient. Coursaget et al. also described particle antigens as a substance containing an "epitopic site" of one of the viral particles. Methods of using these viral particles and the antibodies produced from inoculating chimpanzees and rabbits were also described.

48. Villarejos et al., U.S. Patent 4,702,909 (48), described the isolation of a NANBH antigen, a particle with a size of about 2nm to about 5nm, a density of about 1.24 to about 1.30 g/cc, and contains RNA free or bound to IgG molecules. These particles were isolated from serum of patients diagnosed as having NANBH by isopycnic banding and chromatographic fractionation. The serum sample may be subjected to a prior enzymatic digestion with chymotrypsin or Proteinase K. The fractions having a molecular weight of about 200,000 to 300,000 daltons were found to be antigenic. Antigenicity was determined by ELISA using antibody containing IgG, IgM fractions isolated by Sephadex column chromatography on a serum sample from patients diagnosed with NANAH.

49. Pillot et al., U.S. Patent 4,871,659 (49), describes a method of detecting NANBH antigens by using IgM containing anti-NANBH antibodies. The IgM was purified from the serum of monkeys which have been infected with faeces



obtained from patients who were diagnose with NANBH. The antibodies were then used to detect the presence of antigens associated with NANBH in the faeces of hepatitis patients. No characteristics of the NANBH antigens were described.

50. Wands et al., U.S. Patent 4,870,026 (50), described a purified form of a DNA virus which showed, in chimpanzees, infectivity having the characteristics of NANBH. The DNA virus:

- a. has a molecular weight of greater than  $2 \times 10^6$  daltons;
- b. substantial reactivity toward an anti-HBsAg obtained from cell line ATCC HB 9801;
- c. substantially no immunoreactivity toward an anti-HBsAg obtained from cell line ATCC CRL 8018;
- d. concentration dependent binding capacity toward polyclonal IgG anti-HBsAg which increases with increased concentration of the DNA virus;
- e. forms discrete particulate form in the presence of IgM antibodies from cell line ATCC HB 9801;
- f. a polypeptide profile on SDS polyacrylamide gels, when affinity purified with the IgM antibodies from cell line ATCC HB 9801, comprising bands at about 50,000, about 23,000 and less than about 20,000;
- g. partial homology with HBV DNA by molecular hybridization; and
- h. shows infectivity, in chimpanzees, having the characteristics of NANBH.

Wands et al did not provide any amino acid sequencing of the DNA virus, but describe the use of hybridization techniques to obtain clones of NANBH virus sequences.

51. Chou-Fasman described a predictive model for locating alpha helixes, beta turns and beta sheet structures in a protein.

52. Hopp, U.S. 4,544,101, describes a method of determining the relative greatest local average hydrophility of a protein to locate the epitopes of an antigen. The process calls for assigning relative hydrophilicity values to amino acids and determine the repetitive local average hydrophilicity values along the amino acid sequence of a protein.

The method has not been found to be useful to determine immunodominant epitopes.

53. Shikata, T. et al. EP O,263,761 A2, describes NANB hepatitis antigen having molecular weight of  $44,000 \pm 2,000$  with partial amino acid sequence represented by:

- (i) Tyr-Asn-Ser-Pro-Tur-Asn-Phe-Gln-Ile-Asp-Gly-Arg-Asn-Arg  
Lys;
- (ii) Val-Ile-Met-Asp-Leu-Lys; or
- (iii) Ser-Ser-Phe-Phe-Asn-Ser-Val-Arg-Ser-Val-Phe-Gln-Gly-His-  
Val.

The antigen was obtained from liver specimen with NANBH suing sucrose density gradient and SDS polyarylomide gel electrophoresis.

54. Takahashi, K. et al., EP 0,293,274 A1, describes DNA fragment with base sequence coding for NANBH hepatitis antigen protein. The DNA fragment was obtained from the liver of a human or chimpanzee affected with NANB hepatitis. A cDNA library was constructed. The cDNA was then cloned into a plasmid or lambda t-11 phage and transfected into E.Coli and expressed. The antigen is about 4,400 in MW containing 444 amino acids.

55. Curick, R.J. et al., EP 0,335,135 A2 describe the producing of monoclonal antibodies specific for NANB hepatitis infected liver. The antibodies were produced by isolating lymphocytes from a chimpanzee infected with NANBH. The lymphocytes were cultured. The supernatant of the cell cultures were screened against NANB liver sections. The samples which were positive were rescreened against HBV liver sections. Of these 62 were positive only against NANB liver sections. The 62 cell cultures were again cultured. After six months only 13 cell lines remained stable. These were assayed against NANB liver sections, HBV and HDV infected livers. Of the 13, only one was specific for NANB liver sections alone. This was labelled as a Pt-1.

56. J. Pillot, French Patent Publication No. 2,609,807, discloses a new viral agent which is implicated in NANB hepatitis. A process for detecting the presence of NANB virus utilizes antibodies isolated from the serum of animals which were artificially infected by ingestion of faeces of ANNB hepatist patients. The antibodies IgM are fixed on a solid support for an ELISA procedure.

The patent publication appears to be duplicative of Ref. 49.

57. C. Trepo, U.S. 4,542,016, describes a new vaccine against NANBH virus. The vaccine is prepared from selected serum or plasma of patients suffering from post transfusion hepatitis and asymptomatic patients showing increased level of GPT transaminases (ALT).

The NANB antigen is a vaccinal antigen having the following characteristics:

- a. density between 1.20 and 1.30 g/ml in a CsCl solution and 1.15 and 1.25 g/ml in a sucrose solution.
- b. electrophoretic migration in a alpha-beta-globulin zone;

- c. spherical or filamental particles of about 10 to 45 nm in diameter with the complete virion appearing as a sphere with a dual envelope of about 35 to 45 nm in diameter;
- d. The antigen when administered to an animal provokes the production of antibodies which are reactive with the viral particles or complete virion described in paragraph 3 and is immune reactive with liver tissue sections from patients suffering from NANB. The antibodies are not reactive with HBV particles or liver sections from HBV patients.

No peptides for HCV were described, taught or suggested.

58. Arima, T. et al., Gastroenterologia Jap. 24:540-544 (1989); to clone the HCV (NANBH V), a cDNA library was constructed from RNA extracted from serum of patients with elevated ALT levels and were negative for HBVDNA. 29 clones were obtained which were specific for Japanese HCV. Of these, 12 clones were also found to be specific for American HCV. One of the 12 clones was found to have a unique sequence compared to HAV, HBV and HDV. This clone was identified as clone 18 and has the following predicted amino acid sequence.

Glu-Phe-Gln-Glu-Lys-Lys-Gly-Glu-Ala-Ser-Asn-Gly-Glu-Ala-Glu-Asn-  
Asp-Thr-His-Lys-Lys-Gln-Arg-Arg-Tyr-Lys-Glu-Lys-Glu-Lys-Thr-Ala-  
Thr-Asn-Asn-Pro-Gly-Lys-Asn-Lys-Lys-Pro-Arg-Val-Gly-Arg-Ile-Lys-  
Asn-Trp-Asn-Arg-Glu-Gly-Arg-Lys-Asp-Ala-Tyr-Gln-Ile-Arg-Ile-Arg-  
Arg-Glu-Phe

Clone 18 was tested with serum panels of patients with elevated ALT and negative for HDV. Homology of the nucleotide sequence of the CDNA of clone 18 is not found in human chromosomal DNA, or numerous virii which were

tested. Recombinantly expressed Clone 18 was tested against three serum panels from patients who have been diagnosed to have been infected with HCV. 15 out of 19 samples (79%) with chronic HCV was detected. However, sensitive with acute hepatitis was much less (4/12 = 33%).

59. Arima et al., Gastroenterology Jap., 24: 545-548 (1989), describe the isolation of lambda gt11-random-primed-cDNA clones specific for HCV infection from RNA prepared from pooled serum presumably infected with HCV. 56 clones produced translation products which were positive from the pooled serum. Clone 2, positive only for chronic HCV was further tested. 50% (13/26) of the chronic HCV serum samples were identified. However, none of the acute HCV serum samples were detected. Sequencing of the cDNA gave the predicted amino acid sequence as follows.

Glu-Phe-Pro-Thr-Pro-Arg-Arg-Leu-Gly-Pro-Arg-Leu-Gly-Arg-Arg-Pro-  
Ala-Leu-Met-Ala-Val-Glu-Phe

It has no homology with human chromosomal DNA.

60. Arima et al. Gastroenterology Jap., 24: 685-691 (1989). 29 out of 56 clones reported in Ref. No. 59 were further screened. These were separated into three groups.

- I. Clones 14, 18, 23, 30, 19, 6, 48 and 55  
Positive for both acute and chronic HCV.
- II. Clone 53  
Positive only for acute HCV.
- III. Clones 2, 20, 26, 40, 34, 37, 31, 33, 36, 43, 7, 16  
21, 24, 29, 32, 35, 37, 44 and 45

The nucleotide sequences of these clones ranged from 63 to 280 bp.

Clones 2, 8, 14 and 18 were determined to lack homology to human chromosomal DNA.

Only clones 14, 18, 23, 30, 55, 2, 20, 26, 40, 34, 43 and 45 were specific for HCV in both Japanese and American panels.

Out of 107 samples with elevated ALT and negative HBV, clone 14 detected 14%, clone 2 detected 1.9% and clone 8 detected 4.7% as positive for HCV. It is postulated that clone 8 encodes both envelop and core-like regions of the virus. Whereas, clones 14 and 18 only encodes the latter.

It appears that the infectious agent for transfusion related HCV is different from that of sporadic HCV.

61. Arima et al. Gastroenterology Jap., 25: 218-232 (1990) a lambda gt11-cDNA library was constructed and cloned as described in Ref. Nos. 58, 59 and 60. This paper described one clone, clone 14, specific for HCV. The cDNA of clone 14 was sequenced and its postulated amino acid sequence is as follows:

Glu-Phe-Arg-Glu-Gln-Asp-Gln-Ile-Lys-Thr-Lys-Asp-Arg-Thr-Gln-Gln-  
Arg-Lys-Thr-Lys-Arg-Ser-Thr-Asn-Arg-Arg-Arg-Ser-Lys-Lys-Asn-Glu-  
Lys-Lys-Lys-Lys-Lys-Glu-Phe

The translation product of clone 14 was positive for 22/31 serum samples. It detected 2/2 sporadic acute HCV, 8/9 transfusion associated chronic HCV and 9/10 sporadic chronic HCV. However, it only detected 3/10 transfusion associated acute hepatitis.

62. M. Houghton et al., EP 0, 388,232, this application describes HCV cDNA and polypeptide sequences isolated from pooled serum from a chimpanzee with chronic HCV infection and containing a high titer of the virus.

Numerous peptides, from 5 to 100 amino acids derived from the amino acid sequence of the putative non-structural proteins (See Fig. 17-1) were described. Houghton et al. indicated that some of the peptides were immunogenic while others are not. However, only for the expression products of clones CA279a, CA74a, 13i, CA290a, 33c 40b, 5-1-1, 81, 336, 25c, 14c, 8f, 33f, 33g, 39c, 15e, C100, were described as having HCV antigenicity. Their amino acid sequences of some of these clones were provided in Figs. 1-15.

Houghton et al. suggest that the polypeptides may be useful for diagnostic assays for determining HCV infection by immunoassay procedures. The clones are useful in PCR procedures as probes and the polypeptides may be useful as vaccines.

A review of the amino acid sequences indicate that Peptide II of the present invention is listed among the peptides on page 5 of Houghton et al. In particular, Peptide II corresponds to AA1694-AA1735.

63. G.R. Reyes et al., WO 90/00597, published January 25, 1990. This reference describes post transfusion NANBH virus and antigens isolated from serum or hepatocytes of an infected human or chimpanzee. The beta-gal fusion product of some of the isolated clones were recognized by NANB antiserum.

The virus particles and antigens were isolated by known techniques clone #30 with amino acid sequence as in Fig. 1 was useful of detecting homologous sequences present in the cDNA prepared from documented infectious NANB particles passaged in chimpanzees, but originating in an infected human. Clone #30 has been determined to be 429 nucleotides long with a putative amino acid sequence of 143 amino acids.

Sequences of other clones were reported in Figs. 2-5. However, no data is provided.

64. T. Arima et al., EP O,363,025, published April 11, 1990. This disclosure appears to be duplicative of the four articles published by Arima et al. in Gastroenterologia Jap. See Ref. Nos. 58-61.

This nucleotide sequences of the clones were provided in Fig. 2. The reactive clones appear to be identical to those previously reported giving the same results.

65. B. Seto et al. WO 90/02206, published March 8, 1990. Six clones bearing nucleotide sequences of NANBV were isolated from liver of chimpanzee known to be infected with acute NANBH. These were identified as pSC22, pLC30, pLC50, pSN30, pSN31 and pSN32.

pSC22 was found to hybridize to liver specimens of three NANBH infected chimpanzees. Reaction was observed between 6 to 16 weeks post inoculation. pSC22 did not hybridize with liver specimens from chimpanzees pre-inoculation or infected with HBV.

A quick review of the amino acid sequence of claim 5 does not appear to coincide with the amino acid sequences of peptides I to IX of the present application.

It is to be noted that several of the references are not prior art publications under §102. A review of the dates of publication show that Okamoto et al. (Ref. 27), Reyes et al. (Ref. 63), Arima et al. (Ref. 61), Houghton et al. (Ref. 65) are not prior art references. However, these are included for the Examiner's consideration.




None of the foregoing documents disclose or suggest, independently or in any combination, the invention claimed in the present application. It is applicant's belief that the present invention as claimed is patentable in view of these references.

Respectfully Submitted,

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FORM PTO-1449		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTY. DOCKET NO. 1151-4043		SERIAL NO. 07/558,799	
<b>INFORMATION DISCLOSURE CITATION</b> (Use several sheets if necessary)				APPLICANT    C.Y. Wang			
				FILING DATE    July 26, 1990		GROUP 183	
<b>U.S. PATENT DOCUMENTS</b>							
EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
2.2		4 8 7 9 2 1 2	1/1989	Wang et al.	530	327	
2.2		4 7 3 5 8 9 6	4/1988	Wang et al.	530	327	
<del>2.2</del>	<del>2.2</del>	4 8 3 3 0 7 1	9/1989	Wang et al.	530	327	
<del>2.2</del>		07 2 9 7 6 3 5		<del>Wang et al.</del>			
2.2		4 6 7 3 6 3 4	6/16/87	Seto et al.	530	327	
2.2		4 7 7 7 2 4 5	10/11/88	Foung et al.	530	327	
2.2		4 3 5 6 1 6 4	10/26/82	Tabor et al.	530	327	
2.2		4 3 9 5 3 9 5	7/26/83	Tabor et al.	530	327	
2.2		4 4 6 4 4 7 4	8/7/84	Coursaget et al.	530	327	
2.2		4 7 0 2 9 0 0	10/27/87	Villarejos et al.	530	327	
2.2		4 8 7 1 6 5 9	10/1/89	Pillot	530	327	
<b>FOREIGN PATENT DOCUMENTS</b>							
		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION YES NO
2.2	EP	3 1 8 2 1 6	5/1989	Houghton et al. Europe			
2.2	EP	3 2 8 4 0 3	2/1989	Wang CY Europe			
2.2	EP	2 6 3 7 6 1	4/13/88	Shikata, T. et al. Europe			
2.2	EP	2 9 3 2 7 4	11/31/88	Takahashi, K. et al. Europe			
2.2	EP	3 3 5 1 3 5	10/4/89	Carrick, R.J. et al. Europe			
<b>OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Papers, Etc.)</b>							
2.2		MacCullum FO, et al.: <u>Lancet</u> , 1:6222 (1944).					
2.2		Havens WP: <u>Proc Soc Exp Biol Med</u> , 59:148 (1945).					
EXAMINER				DATE CONSIDERED			
L. Lu				1/30/91			
EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.							

FORM PTO-1449

U.S. DEPARTMENT OF COMMERCE  
PATENT AND TRADEMARK OFFICEATTY. DOCKET NO.  
1151-4043SERIAL NO.  
07/558,799

## INFORMATION DISCLOSURE CITATION

(Use several sheets if necessary)

APPLICANT C.Y. Wang

FILING DATE July 26, 1990

GROUP 183

## U.S. PATENT DOCUMENTS

EXAMINER INITIAL	DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE
22	4 5 4 2 0 1 6	9/7/85	Trepo	530	327	
22	4 5 5 4 1 0 1	11/19/85	Hopp	530	327	

## FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	CLASS	SUBCLASS	TRANSLATION	
							YES	NO
22	FR	FR 2 6 0 9 8 0 7	7/22/88	Pilote FRANCE				
22	EP	EP 3 8 8 2 3 2	9/18/90	Houghton et al. Europe				
22	WO	WO 90 0 0 5 9 7	1/25/90	Reyes et al. Europe				
22	EP	EP 3 6 3 0 2 5	4/11/90	Arima et al. Europe				
22	WO	WO 90 02 2 2 0 6	3/8/90	Seto, B. et al. Europe				

## OTHER DOCUMENTS (Including Author, Title, Date, Pertinent Papers, Etc.)

22		Krugman S, et al.: JAMA, 200:365 (1967).
22		Prince AM: Lancet, 2:241 (1974).

EXAMINER

J. Lee

DATE CONSIDERED

7/30/91

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

ATTY. DOCKET NO.  
1151-4043

**SERIAL NO. 07/558,799**

APPLICANT C.Y. Wang

**FILING DATE** July 26, 1990

**GROUP 183**

[illegible][illegible]

J2.		Alter HJ, et al: <u>Lancet</u> , 2:838 (1975).
212'		Gaibrainh RM, et al: <u>Lancet</u> , 2:886 (1975).

**EXAMINER**

**DATE CONSIDERED**

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

U.S. DEPARTMENT OF COMMERCE  
PATENT AND TRADEMARK OFFICE

**SERIAL NO. 07/558,799**

APPLICANT C.Y. Wang

FILING DATE July 26, 1990

**GROUP 183**

[illegible][illegible]

OTHER DOCUMENTS (including Author, Title, Date, Pertinent Papers, Etc.)			
22			Mosley JW, et al: <u>N. Engl J Med</u> , 296:75 (1977).
22			Dienstag JL: <u>Rush-Presbyterian-St. Luke's Med Bull</u> , 15:104 (1976).

J. Lee

DATE CONSIDERED 1/30/91

FORM PTO-FB-AB20  
(also form PTO-1449)

22

FORM PTO-FB-AB20  
(also form PTO-1449)

FORM PTO-FB-AB20  
(also form PTO-1449)

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(also form PTO-1449)

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22

ATTY. DOCKET NO.  
1151-4043

SERIAL NO. 07/558,799

### INFORMATION DISCLOSURE CITATION

*(Use several sheets if necessary)*

APPLICANT C.Y. Wang

FILING DATE July 26, 1990

**GROUP 183**

## U.S. PATENT DOCUMENTS

[illegible]

## FOREIGN PATENT DOCUMENTS

[illegible]

**OTHER DOCUMENTS** (Including Author, Title, Date, Pertinent Papers, Etc.)

22			Kubo, Y., et al: <u>Nucleu Acid Res.</u> 17:10367-10372 (1989).
22			Slide Presentation by Abbot Laboratories to American Association of Blood Banks (October 1989).

**EXAMINER**

L. Lee

**DATE CONSIDERED**

1/30/21

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

**FORM PTO-FB-AB20**  
(also form PTO-1449)

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(also form PTO-1449)

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FORM PTO-FB-AB20  
(also form PTO-1449)

FORM PTO-1449

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PATENT AND TRADEMARK OFFICE

ATTY. DOCKET NO.  
1151-4043

**SERIAL NO. 07/558,799**

### INFORMATION DISCLOSURE CITATION

*(Use several sheets if necessary)*

APPLICANT C.Y. Wang

FILING DATE July 26, 1990

**GROUP 183**

## U.S. PATENT DOCUMENTS

[illegible]

## FOREIGN PATENT DOCUMENTS

[illegible]

**OTHER DOCUMENTS** (Including Author, Title, Date, Pertinent Papers, Etc.)

22			Arima T. et al., <u>Gastroenterologia Jap.</u> , 24:685-691 (1989).
22			Arima T. et al., <u>Gastroenterologia Jap.</u> , 25:2218-232 (1990).

**EXAMINER**

**DATE CONSIDERED**

EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609; Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.



1151-4043

PATENT  
U.S.S.N. 07/558,799

#31a  
S. K. Lee  
1-23-91

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Chang Yi Wang  
Serial No. : 07/558,799  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE  
DETECTION OF ANTIBODIES TO HCV, DIAGNOSIS  
OF HCV INFECTION AND PREVENTION THEREOF  
AS VACCINES  
Group Art Unit : 183  
Examiner : Lester Lee

Hon. Commissioner of Patents and Trademarks  
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please amend the application as follows.

In The Specification

Page 16, line 5: delete "Ser-Cys" between "Glu-Glu" and "Gln-His" and insert

therefor -- Cys-Ser --

line 26: delete "His" at the beginning of the line, before "Thr-Asn" and

insert therefor -- Asn --.

✓ Page 17, line 2: delete "Thr" between "Pro-Leu" and "Gly-Asn" and insert

therefor -- Tyr --.

Page 18, line 25: delete "Ser-Cys" between "Glu-Glu" and "Gln-His" and insert therefor -- Cys-Ser --.

Page 19, line 16-17: delete "His" at the beginning of the line, before "Thr-Asn" and insert therefor -- Asn --.

line 20-21: delete "Thr" between "Pro-Leu" and "Gly-Asn" and insert therefor -- Tyr --.

Page 24, line 7: delete "VIIE" and insert therefor -- VIIIE --.

Page 26, line 3: delete "Ser-Cys" between "Glu-Glu" and "Gln-His" and insert therefor -- Cys-Ser --.

line 24: delete "His" at the beginning of the line, before "Thr-Asn" and insert therefor -- Asn --.

line 28: delete "Thr" between "Pro-Leu" and "Gly-Asn" and insert therefor -- Tyr --.

Page 28, line 20: delete "Ser-Cys" between "Glu-Glu" and "Gln-His" and insert therefor -- Cys-Ser --.

Page 29, line 11-12: delete "His" at the beginning of the line before "Thr-Asn" and insert therefor -- Asn --.

line 15-16: delete "Thr" between "Pro-Leu" and "Gly-Asn" and insert therefor -- Tyr --.

line 22-23: delete "Pre" between "Gln" and "Lys-Gln" and insert



therefor -- Phe --.

line 23: delete "Arg" at the beginning of the line before "Glu-Val" and  
insert therefor -- Ala --.

Page 30, line 4-5: delete "His" between "Lys-Arg" and "Thr-Asn" and insert  
therefor -- Asn --.

line 7: delete "Thr" between "Pro-Leu" and "Gly-Asn" and insert  
therefor -- Tyr --.

Page 31, Table 1, first column on left: delete "VD" and insert therefor -- VC --;  
and delete "VC" and insert therefor -- VD --.

Page 34, line 1: delete "His" at end of the line after "Lys-Arg" and insert  
therefor -- Asn --.

✓ line 4: delete "Gly-Arg-Arg-Gln-Pro" at the beginning of the line  
before "Ile-Pro".

✓ line 5: delete "Thr" at the beginning of the line before "Gly-Asn" and  
insert therefor -- Tyr --.

Page 35, line 10-11: delete "Ser-Cys" between "Glu-Glu" and "Gln-His" and  
insert therefor -- Cys-Ser --.

Page 36, line 1-2: delete "His" at the beginning of the line before "Thr-Asn" and  
insert therefor -- Asn --.

line 5-6: delete "Thr" between "Pro-Leu" and "Gly-Asn" and insert

therefor -- Tyr --.

Page 38, line 4: insert between "namely" and "LAEQF" -- Leu-Ala-Glu-Gln-Phe -- and insert -- ( -- and -- ) -- before and after "LAEQF".

line 5: insert before "HLPYI" -- His-Leu-Pro-Tyr-Ile -- and insert -- ( -- and -- ) -- before and after "HLPYI".

line 7: insert before "EECSQHLPYI" -- Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile -- and insert -- ( -- and -- ) -- before and after "EECSQHLPYI".

line 9: insert before "SGKPAIIPDR" -- Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg -- and insert -- ( -- and -- ) -- before and after "SGKPAIIPDR".

line 10: insert before "GLLQT" -- Gly-Leu-Leu-Gln-Thr -- and insert -- ( -- and -- ) -- before and after "GLLQT".

line 11: insert before "EVIAP" -- Glu-Val-Ile-Ala-Pro -- and insert -- ( -- and -- ) -- before and after "EVIAP".

line 22: insert before "SGKPA" -- Ser-Gly-Lys-Pro-Ala -- and insert -- ( -- and -- ) -- before and after "SGKPA"; insert before "IIPDREV" -- Ile-Ile-Pro-Pro-Asp-Arg-Glu-Val -- and insert -- ( -- and -- ) -- before and after "IIPDREV".

Page 49, line 1: insert -- > -- before "100 I.U./L".

line 9: delete "n=270" and insert therefor -- n=370 --.

Page 56, line 3: insert -- < -- before the first instance of "20" and insert

-- > -- before the second instance of "20".

Page 70, line 15: delete ")" after "VIIIIE".

Page 71, line 12: delete "VIII" and insert therefor -- VIIIIE --.

Page 75, line 6: insert -- ~ -- between "O.D." and "1.5".

Page 76, Table 8: fourth column, below "ALT", delete "mu/mL" and insert therefor -- I.U./L --; fifth column, below "AST" delete "mu/mL" and insert therefor -- I.U./L --.

Page 77 & 78, Table 9: last column on right, insert -- > before "25 i.u./L" and correct "i.u." to -- IU --.

#### In the Claims

Claim 1 (i), line 1: delete "Ser-Cys" between "Glu-Glu" and "Gln-His" and insert therefor -- Cys-Ser --.

(ix), line 2: delete "His" at the beginning of the line before "Thr-Asn" and insert therefor -- Asn --.

(x), line 3: delete "Thr" between "Pro-Leu" and "Gly-Asn" and insert therefor -- Tyr --.

REMARKS

The specification and claims have been carefully reviewed and the typographical and clerical errors corrected. Support for the amendment of pages 16, 17, 18, 19, 26, 28, 29, 35, 36, and Claim 1 can be found in Table 1, Fig. 1-1, and Table 7, Fig 11-1 as originally filed. Support for the amendment of pages 24 and 71 can be found in the specification at page 24, line 24, and Example 17. Support for the amendment on page 34 is to be found in Table 7, Fig. 11-1, Fig. 11-2, and Claim 7 as originally filed.


Page 38 was amended to put in the three letter codes for the amino acid sequences. The amendment on page 49 is to correct an obvious typographical error. Support for the amendment of page 75 can be found in Table 9.

No new matter has been introduced by the amendments. Entry of the amendment is requested.

Respectfully Submitted,

MORGAN & FINNEGAN

Dated: December 7, 1990

By:   
Maria C.H. Lin  
Registration No. 29,323

Mailing Address:

MORGAN & FINNEGAN  
345 Park Avenue  
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(212) 751-6849 Telecopier

## File History Report

☐ Paper number \_\_\_\_\_ is missing from the United States Patent and Trademark Office's original copy of the file history. No additional information is available.

☒ The following page(s) Attachment of paper number 3 is/are missing from the United States Patent and Trademark Office's original copy of the file history. No additional information is available

Additional comments: \_\_\_\_\_



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	INVENTOR	ATTORNEY
07/558,799	07/26/90	WANG	LEE, L

MORGAN & FINNEGAN  
345 PARK AVE.  
NEW YORK, NY 10154

189  
DATE MAILED:

02/11/91

This is a communication from the examiner in charge of your application.  
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☐ Responsive to communication filed on \_\_\_\_\_ ☐ This action is made final.  
A shortened statutory period for response to this action is set to expire 3 month(s), \_\_\_\_\_ days from the date of this letter.  
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☒ Notice re Patent Drawing, PTO-948.
3. ☒ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, Form PTO-152
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ \_\_\_\_\_

Part II SUMMARY OF ACTION

1. ☒ Claims 1-43 are pending in the application.  
Of the above, claims 12-27 are withdrawn from consideration.
2. ☐ Claims \_\_\_\_\_ have been cancelled.
3. ☐ Claims \_\_\_\_\_ are allowed.
4. ☒ Claims 1-11 and 28-43 are rejected.
5. ☐ Claims \_\_\_\_\_ are objected to.
6. ☐ Claims \_\_\_\_\_ are subject to restriction or election requirement.
7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on \_\_\_\_\_ Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on \_\_\_\_\_, has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed \_\_\_\_\_, has been ☐ approved; ☐ disapproved (see explanation).
12. ☐ Acknowledgement is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. \_\_\_\_\_; filed on \_\_\_\_\_.
13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION

Serial No. 558799

-2-

Art Unit 189B

The Group and/or Art Unit location of your application in the PTO has changed. To aid in correlating any papers for this application, all further correspondence regarding this application should be directed to Group Art Unit 189B.

Restriction to one of the following inventions is required under 35 U.S.C. § 121:

I. Claims 1-11 and 28-43, drawn to peptides and kits, classified in Class 530, subclass 324.

II. Claims 12-26, drawn to method of assaying, classified in Class 435, subclass 7.

III. Claim 27, drawn to antibodies to HCV or NANBHV, classified in Class 424, subclass 88.

The inventions are distinct, each from the other because of the following reasons:

Inventions I and II are related as product and process of use. The inventions can be shown to be distinct if either or both of the following can be shown: (1) the process for using the product as claimed can be practiced with another materially different product or (2) the product as claimed can be used in a materially different process of using that product (M.P.E.P. § 806.05(h)). In the instant case the peptides of Group I can be used to prepare a vaccine.

Group I is distinct from Group III since the peptide can be used to in a method of assaying.

Serial No. 558799

-3-

Art Unit 189B

Because these inventions are distinct for the reasons given above and have acquired a separate status in the art as shown by their different classification, have acquired a separate status in the art because of their recognized divergent subject matter and the search for Group I is not required for Groups II and III, restriction for examination purposes as indicated is proper.

During a telephone conversation with Maria C. H. Lin on November 29, 1990 a provisional election was made without traverse to prosecute the invention of Group I, claims 1-11 and 28-43. Affirmation of this election must be made by applicant in responding to this Office action. Claims 12-27 withdrawn from further consideration by the Examiner, 37 C.F.R. § 1.142(b), as being drawn to a non-elected invention.

Claims 1-11 and 28-43 rejected under 35 U.S.C. § 112, first paragraph, as the disclosure is enabling only for claims limited to the specific peptides of the examples since these are the only ones applicant has shown to be effective in detecting antibodies to hepatitis C virus. See M.P.E.P. §§ 706.03(n) and 706.03(z).

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.



Serial No. 558799

-4-

Art Unit 189B

The specification is objected to under 35 U.S.C. § 112, first paragraph, as failing to adequately teach how to make and/or use the invention, i.e. failing to provide an enabling disclosure.

The terms "analogues, segments, mixtures, combinations, conjugates and polymers thereof" in the specification and claims are so vague and indefinite as to require undue experimentation of one skilled in the art to determine the meets and bounds of said terms. Each possible peptide would have to be prepared and tested to determine whether effective in detecting antibodies to hepatitis C virus and this would require undue experimentation of one skilled in the art.

Claims 1-11 and 28-43 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification.

Claims 1-11 and 28-43 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The claims are rejected for the following reasons.

- (a) the term "analogues, segments, mixtures, combinations, conjugates and polymers thereof" fail to particularly point out and distinctly claim the peptides included in the claims,

Serial No. 558799

-5-

Art Unit 189B

(b) the peptides of claims 2-11 and 29-42 do not have antecedent basis in the generic claim,

(c) claim 43 is rejected as failing to define the other amino acids which can be present in the peptide.

The references cited on the enclosed PTOL 892 are to show the state of the art.

Any inquiry concerning this communication should be directed to Lester L. Lee at telephone number (703)308-3997.

Lee/bg  
February 06, 1991

*Lester L. Lee*  
LESTER L. LEE  
PRIMARY PATENT EXAMINER  
ART UNIT 189B  
189B

TO SEPARATE, HOLD TOP AND BOTTOM EDGES, SNAP-APART AND DISCARD CARBON

FORM PTO-892 (REV. 3-78)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		SERIAL NO. <b>07/558799</b>	GROUP ART UNIT <b>189B</b>	ATTACHMENT TO PAPER NUMBER <b>4</b>					
NOTICE OF REFERENCES CITED				APPLICANT(S) <b>Wang</b>							
U.S. PATENT DOCUMENTS											
•		DOCUMENT NO.			DATE	NAME	CLASS	SUB- CLASS	FILING DATE IF APPROPRIATE		
	A	4591552			5/86	Neurath	530	326			
	B	4596674			6/86	Emini et al.	530	326			
	C	4599230			7/86	Milich et al.	530	326			
	D	4599231			7/86	Milich et al.	530	326			
	E	4818527			4/89	Thornton et al.	530	326			
	F	4879212			11/89	Wang et al.	530	326			
	G	4879213			11/89	Fox et al.	530	326			
	H	4882145			11/89	Thornton et al.	530	324			
	I										
	J										
	K										
FOREIGN PATENT DOCUMENTS											
•		DOCUMENT NO.			DATE	COUNTRY	NAME	CLASS	SUB- CLASS	PERTINENT SHTS. DWG.	PP. SPEC.
	L										
	M										
	N										
	O										
	P										
	Q										
OTHER REFERENCES (Including Author, Title, Date, Pertinent Pages, Etc.)											
	R										
	S										
	T										
	U										
EXAMINER		DATE									
<b>Leslie L. Lee</b>		<b>1/31/91</b>									
* A copy of this reference is not being furnished with this office action. (See Manual of Patent Examining Procedure, section 707.05 (a).)											

PTO - 948  
(Rev. 8-82)

U.S. DEPARTMENT OF COMMERCE  
PATENT AND TRADEMARK OFFICE

ATTACHMENT TO PAPER NUMBER	4
S.N.	558799

GROUP

PTO draftsmen now review both  
formal and informal drawings.  
The examiner will require  
submission of formal drawings  
at the appropriate time.

### NOTICE OF PATENT DRAWING OBJECTION

Drawing Corrections and/or new drawings may only be  
submitted in the manner set forth in the attached letter,  
"Information on How to Effect Drawing Changes," PTO 4573.

A. ☒ The drawings, filed on 7/26/90, are objected to as informal for reason(s)  
checked below:

1. ☐ Lines Faint.

11. ☐ Section Must Be Hatched

2. ☐ Paper Poor.

12. ☐ Drawing Unintelligible.

3. ☒ Numerals Poor. Final must be at least  
fig 1-13(6) 1/8" tall

13. ☒ Signs, Legends, Pack  
figs 1-15(2)

4. ☒ Lines Rough and Marred.  
figs 1-7(1)

14. ☐ Photographs.

5. ☐ Shade Lines Required.

15. ☐ Drawing Unintelligible.  
[See 37 CFR 1.83 (1)]

6. ☐ Figures Must Be Numbered.

16. ☐ Drawing Not on 8 1/2" x 14",  
or 11" x 17.5 cm. required.

7. ☐ Heading Space Required.  
8(1), 8(2)

8. ☐ Figures Must Not Be Connected.

17. ☐ Drawing Dimensions Required:  
[Left 2.5 cm. [Right 1.5 cm.  
[Top 2.5 cm. [Bottom 1.0 cm.

9. ☐ Cross-Cross Hatching Objectionable.

10. ☐ Double-Line Hatching Objectionable.

18. ☐ Drawing

B. ☒ The drawings, submitted on 7/26/90, are so informal they cannot be  
corrected. New drawings are required. Submission of the new drawings must be  
made in accordance with the attached letter.



## PATENT

Docket No. 1151-4043

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s): Chang Yi Wang      Group Art Unit: 189  
Serial No. : 07/558,799      Examiner: Lester Lee  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV,  
DIAGNOSIS OF HCV INFECTION AND PREVENTION THEREOF AS VACCINES

## PETITION AND FEE FOR EXTENSION OF TIME (37 C.F.R. § 1.136(a))

HON. COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

Sir:

1. This is a petition for an extension of time for filing an Amendment and Response Pursuant to 35 C.F.R. Sec. 1.115 to February 11, 1990 Office
2. The communication in connection with the matter for which this extension Action. is requested

☒ is filed herewith.

☐ has been filed on \_\_\_\_\_.

3. ☒ Applicant is a small entity--verified statement is attached ☐, or has already been filed ☒.

4.	Total Months Requested	Fee for Other than Small Entity	Fee for Small Entity
a. <input checked="" type="checkbox"/>	one month	\$100.00	\$50.00
b. <input type="checkbox"/>	two months	\$300.00	\$150.00
c. <input type="checkbox"/>	three months	\$730.00	\$365.00
d. <input type="checkbox"/>	four months	\$1150.00	\$575.00
		Fee \$ 50.00	

- e. ☐ An extension for \_\_\_\_\_ months has already been secured for filing the above-identified communication and the fee paid therefor of \$\_\_\_\_\_ is deducted from the total fee due for the total months of extension now requested. The fee for this extension (\$\_\_\_\_\_), minus the fee previously paid (\$\_\_\_\_\_), equals \$\_\_\_\_\_ (total fee due).

5. ☒ A Check in the amount of \$ 50.00 to cover the extension fee is attached.
6. ☐ Charge fee to Deposit Account No. 13-4500. Order No. \_\_\_\_\_. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.
7. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required by this paper, or credit any overpayment to Deposit Account No. 13-4500. Order No. 1151-4043 A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted,

MORGAN &amp; FINNEGAN

Dated: May 22, 1991

By: Maria G.H. Lin

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070 AA 05/31/91 07554799

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PATENT  
U.S.S.N. 07/558,799

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Examiner : Lester Lee

Hon. Commissioner of Patents and Trademarks  
Washington, D.C. 20231

RECEIVED GROUP 180

JUN 03 1991

AMENDMENT AND RESPONSE PURSUANT to 35 C.F.R. §1.115

Sir:

This is in response to the office action dated  
February 11, 1991 for which a response period of three  
months was set. Applicant hereby requests an extension of  
one month. Enclosed herewith is the fee therefor.

AMENDMENT

Please amend the application as follows.

In the Specification

Page 5, line 25: delete "SOD/HVC C-100" and  
insert therefor -- SOD/HCV C100-3 --.

Page 20, line 29: delete "SOD/HCV C-100" and  
insert therefor -- SOD/HCV C100-3 --.

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Page 21, lines 25 & 30: delete "SOD/HCV C-100"  
and insert therefor -- SOD/HCV C100-3 --.

Page 22, line 1: delete "SOD/HCV C-100" and  
insert therefor -- SOD/HCV C100-3 --.

Page 40, Heading of Table 2: delete "Analogues"  
and insert therefor -- Segments --.

Page 41, line 9: delete "SOD/HCV C-100" and  
insert therefor -- SOD/HCV C100-3 --.

Page 48, line 17: delete "IID" at end of line and  
insert therefor -- IIID --.

Page 49, lines 13 & 24: delete "SOD/HCV C-100"  
and insert therefor -- SOD/HCV C100-3 --.

Page 63, line 23: delete "2" after "Table" and  
insert therefor -- 1 --.

In the Claims

Delete claims 2, 4, 7, 29, 34 and amend Claims 1,  
3-6, 8, 9, 28 and 43 as follows.

Sub 2  
D'BI  
1 (Amended). A peptide composition comprising a  
peptide [with an amino acid sequence] selected from the  
group consisting of Peptide I to IX each peptide with an  
amino acid sequence as follows:

- (i) Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X  
(Peptide I)
- (ii) [Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-  
Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-  
His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-  
Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-  
Gly-Leu-X  
(Peptide II)
- (iii)] Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-X  
(Peptide II[H])
- ([iv]iii) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-  
Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X  
(Peptide III)
- (iv) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X  
(Peptide IV)
- (v[i]) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-  
Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-  
Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X  
(Peptide V)
- (vi[i]) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-  
Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-  
Thr-X  
(Peptide VI)
- (vii[i]) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-  
Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-  
Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-  
Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-  
Val-Ser-Pro-X  
(Peptide VII)
- ([ix]viii) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-  
Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-  
Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-  
Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-  
Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-  
Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-  
Arg-X[, and]  
(Peptide VIII)



and

(ix) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X  
(Peptide IX)

wherein X is -OH or -NH<sub>2</sub>; and

(x[i]) analogues, segments, mixtures,  
[combinations,] conjugates and polymers  
thereof.

3. A peptide composition according to Claim 1  
wherein the peptide comprises[ing] a segment of Peptide II  
and has[ving] an amino acid sequence selected from the group  
consisting of:

- (i) Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IIC)
- (ii) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IID)
- (iii) Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IIE)
- (iv) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IIF)
- (v) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IIG)

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[(vi) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;]

wherein X is -OH or -NH<sub>2</sub> and analogues thereof.

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as

5. A peptide composition according to Claim 1 wherein the peptide comprises[ing] a segment of Peptide III and has[ving] an amino acid sequence selected from the group consisting of:

(i) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

(Peptide IIIC)

(ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

(Peptide IIID)

(iii) Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

(Peptide IIIE)

[(iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;]

wherein X is - OH or -NH<sub>2</sub> and analogues thereof.

B3

6. A peptide composition according to Claim 5 wherein the peptide [and] has[ving] an amino acid sequence as follows:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

(Peptide IIID)

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

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8. A peptide composition according to Claim 1 wherein the peptide comprises[ing] a segment of Peptide VIII and has[ving] an amino acid sequence selected from the group consisting of:

- (i) Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIIID)
- (ii) Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIIIC)
- (iii) Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIIIB)
- (iv) Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIIIA)

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

9. A peptide composition according to Claim 1 wherein the peptide comprises[ing] a segment of Peptide IX and has[ving] an amino acid sequence selected from the group consisting of:

- (i) Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;  
(Peptide IXD)
- (ii) Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;  
(Peptide IXC)

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(iii) Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

(Peptide IXB)

(iv) Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

(Peptide IXA)

wherein X is -OH or -NH<sub>2</sub> or an analogue thereof.

B5

28. An enzyme linked immunosorbent assay (ELISA) test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

- (i) a solid substrate [compartmented enclosure containing multiple wells] coated with a peptide composition according to Claim 1;
- (ii) a negative control sample;
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent [comprising PBS buffer containing 20% by volume normal goat serum; 1% by weight gelatin and 0.05% by weight TWEEN 20];
- (v) [peroxidase] enzyme labelled antibodies to human IgG; and
- (vi) [a color change indicator] an enzyme substrate.

B6

43 (Amended). A peptide which is immunoreactive to HCV with at least about 15 to not more than about 65 amino acids [in a sequence having as a segment thereof said segment] containing an epitope selected from the group consisting of :

- (i) Ser-Gly-Lys-Pro-Ala;
- (ii) Ile-Ile-Pro-Asp-Arg;
- (iii) Glu-Glu-Cys-Ser-Gln;
- (iv) His-Leu-Pro-Tyr-Ile;
- (v) Glu-Gln-Gly-Met-Met;

- B7C
- (vi) Leu-Ala-Glu-Gln-Phe;
  - (vii) Lys-Gln-Lys-Ala-Leu;
  - (viii) Gly-Leu-Leu-Gln-Thr;
  - (ix) Glu-Val-Ile-Ala-Pro; and
  - (x) Glu-Val-Leu-Tyr-Arg;

and wherein the remaining amino acids in the peptide substantially correspond to the amino acid sequence of the segment of the HCV containing one of the said epitopes.

Please add the following new claims.

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44. A peptide Having the amino acid sequence:  
Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-  
Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-  
Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-  
Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X

wherein X is -OH or -NH<sub>2</sub>. (Peptide II)

24  
45. A peptide having the amino acid sequence:  
Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-  
Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-  
Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-  
Leu-Pro-Tyr-Ile-X

wherein X is -OH or -NH<sub>2</sub>. (Peptide III)

25  
46. A peptide having the amino acid sequence:  
Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-  
Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-  
Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-  
Ala-Glu-Gln-Phe-X

wherein X is -OH or -NH<sub>2</sub>. (Peptide IV)

~~47.~~<sup>24</sup> A peptide having the amino acid sequence:  
Lys-Gly-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-  
Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-  
Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-  
Met-Trp-Asn-Phe-X

wherein X is -OH or NH<sub>2</sub>. (Peptide V)

~~48.~~<sup>27</sup> A peptide having the amino acid sequence:  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-  
Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-  
Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-  
Gln-Lys-Leu-Glu-Thr-X

wherein X is -OH or -NH<sub>2</sub>. (Peptide VI)

~~49.~~<sup>28</sup> A peptide having the amino acid sequence:  
Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-  
Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-  
Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-  
Arg-Gly-Asn-His-Val-Ser-Pro-X

wherein X is -OH or NH<sub>2</sub>. (Peptide VII)

~~50.~~<sup>29</sup> A peptide having the amino acid sequence:  
Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-  
Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-  
Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-  
Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-  
Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-  
Arg-X

wherein X is -OH or -NH<sub>2</sub>. (Peptide VIII)

51. <sup>30</sup> A peptide having the amino acid sequence:

✓ Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-  
✓ Glu-Gly-Arg-Tyr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-  
✓ Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-  
Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-  
Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-  
Gly-X

wherein X is -OH or -NH<sub>2</sub>. (Peptide IX)

52. <sup>31</sup> A peptide having the amino acid sequence:

Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-  
Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-  
Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-  
Ala-Leu-Gly-Leu-X

wherein X is -OH or -NH<sub>2</sub>. (Peptide IIF)

53. <sup>32</sup> A peptide having the amino acid sequence:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-  
Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-  
Gln-His-Leu-Pro-Tyr-Ile-X

wherein X is -OH or -NH<sub>2</sub>. (Peptide IIID)

54. <sup>33</sup> A peptide having the amino acid sequence:

Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-  
Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-  
Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-  
Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-  
Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

wherein X is -OH or NH<sub>2</sub>. (Peptide IXD)

55. An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

- (i) a solid substrate coated with a peptide composition containing any one of the following peptides: I, IIF, II, III, IIID, IV, V, VI, VII, VIII, and IXD or mixtures thereof;
- (ii) a negative control sample; and
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.

56. An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

- (i) according to claim 55, wherein the peptide composition comprises Peptide II;
- (ii) a negative control sample; and
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.



57. An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

- (i) according to claim 55, wherein the peptide compositions comprises Peptide V;
- (ii) a negative control sample; and
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.

58. An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

- (i) according to claim 55, wherein the peptide compositions comprises Peptide VII;
- (ii) a negative control sample; and
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.

59. An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

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- (i) wherein the peptide composition comprises a mixture of Peptides II and V;
  - (ii) a negative control sample; and
  - (iii) an inactivated HCV positive control sample;
  - (iv) specimen diluent;
  - (v) enzyme labelled antibodies to human IgG; and
  - (vi) an enzyme substrate.

60. An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

- (i) wherein the peptide composition comprises a mixture of Peptides II and VIII;
- (ii) a negative control sample; and
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.

61. An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

- (i) wherein the peptide composition comprises a mixture of Peptides II, V and VIII;
- (ii) a negative control sample; and

- Sub  
B7C  
Concl
- (iii) an inactivated HCV positive control sample;
  - (iv) specimen diluent;
  - (v) enzyme labelled antibodies to human IgG; and
  - (vi) an enzyme substrate.

Please cancel Claims 12 to 27 without prejudice.

REMARKS

The specification has been reviewed and amended to correct typographical and clerical errors. No new matter has been introduced.

Claims 1-9 have been amended to provide antecedent basis for the dependent claims as suggested by the Examiner. Support for the amendment is found in the original claims as filed. Claims 28-42 have been amended to delete unnecessary details. Claim 43 has been amended to more clearly and distinctly claim the invention. Support for the amendment can be found in the specification, Example 1 on Page 44-46, Table I, Page 31 and specifically on Pages 37-38 and Figs. 1-1, 1-2, 1-3 and 1-4. No new matter has been introduced.

New Claims 44-61 are added. Support for new Claims 44 to 50 can be found in originally filed Claim 1. Support for new Claims 51 to 61 can be found in the specification as follows:

<u>Claim</u>	<u>Support in Specification</u>
51	Page 32, Table 7.

<u>Claim</u>	<u>Support in Specification</u>
52	Page 31, Table I; Page 33, lines 7-20.
53	ditto
54	Page 32, Table 7; Page 33, line 26 to Page 34, line 8.
55-61	Example 1, Pages 44-46 and Table 1, Page 31; Example 14, Pages 68-69; Table 7, Page 32; Example 2, Pages 46-54; and Example 12, Pages 64-65.

Claims 12-27 are canceled without prejudice to file a divisional application therefor. No new matter has been entered. Entry of the amendment is requested.

The Examiner has imposed restriction of the claims to:

Group I. Claims 1-11 and 28-43, drawn to peptides and kits, classified in Class 530, subclass 324.

Group II. Claims 12-26, drawn to methods of assaying classified in Class 435, subclass 7.

Group III. Claim 27, drawn to antibodies to HCV or NANBV, classified in Class 424, subclass 88.

Applicant elects Claims 1-11 and 28-43. However, Applicant wish to point out that Claims 28-42 directed to the peptide test kits uses the assay methods of Claims 12-26 and should be examined together. The Examiner indicated that the method of assay is in a separate group and should be examined separately. Reconsideration of the restriction

requirement is requested. To have claims directed to immunoassay methods that are employed in the ELISA kits examined separately appear to be contrary to the policy of not granting two patents for essentially the same invention.

With the present amendment, the claims now pending are Claims 1-11, 28-61. Newly added Claims 44-61 are directed to specific peptides and mixtures used in the ELISA test kits which are fully supported by the specification. Therefore, it is believed that the rejections do not apply to these claims. The Examiner did not apply any references to the Claims. It is, therefore, believed that these newly added claims are allowable.

The Examiner rejected Claims 1-11 and 28-43 under 35 U.S.C. §112, first paragraph. It is the Examiner's position that the disclosure is enabling only for claims limited to the specific peptides of the examples, "since these are the only one[s] applicant has shown to be effective in detecting antibodies to hepatitis C virus".

The Examiner stated that:

The terms "analogues, segments, mixtures, combinations, conjugates and polymers thereof" in the specification and claims are so vague and indefinite as to require undue experimentation of one skilled in the art to determine the metes and bounds of said terms. Each peptide would have to be prepared and tested to determine whether effective in detecting antibodies to hepatitis C virus and this would require undue experimentation of one skilled in the art.

Reconsideration for the rejection is requested for the following reasons.

Under 35 U.S.C. §112, first paragraph, the dispositive issue is whether applicants disclosure,

considering the level of ordinary skill in the art as of the date of the application, would have enabled a person of such skill to make and use the applicant's invention without undue experimentation. See U.S. v. Telectronics, Inc., USPQ 2d 1217, 1222 (Fed. Cir. 1988). This standard is also applicable to exparte applications. In re Meyers, 161 USPQ 668, 671 (CCPA 1969).

Firstly, the claims are directed to the use of synthetic peptides in immunoreactions for diagnostic tests. In this technology, the level of ordinary skill in the art is very high. It is understood by those of skill in the art that whether a synthetic peptide will have immunoreactivity for detecting antibodies to a particular pathogen is unpredictable. The specific region(s) of the protein structure of the pathogen must be carefully analyzed and by an approach the Applicant and others have termed "site directed serology", the immunodominant epitopes of the surface of proteins of the pathogen is mapped and determined.

Once the immunodominant enpitopes for the particular antigen are determined, then synthetic peptides containing the epitopes can be constructed and it is understood that the synthetic peptides containing such epitopes will have immunoreactivity to the antibodies to the particular antigen, which in the present case is HCV.

With this understanding, the specification clearly describes how analogues of the claimed sequences can be made. On page 6, line 6 to page 7, line 2, the specification clearly teaches how analogues of the claimed sequences can be made by examining the predicted amino acid

sequences of the various strains of the HCV. For example, the amino acid sequences of the known existing strains of HCV are J-1, J-4 and prototype PT. From the predicted amino acid sequence for the various strains of the virus, the corresponding regions containing the immunodominant epitopes as described and claimed by the Applicant can be determined and analogues constructed. Further, it is known to those of skill in the art that conservative substitutions and deletions of amino acids in a peptide sequence do not cause the loss of activity in a peptide. For example, the substitution of valine for alanine or glycine, is known to be permitted. This is described on Page 27, lines 5 to 16. Thus, to one with skill in this art, it is clear that analogues immunoreactive to antibodies to HCV be constructed. Therefore, the specification is enabling.

It is true that working examples showing test results of analogues of the claimed peptides have not been presented. However, it has long been recognized by the Board of Patent Appeals and Interferences and the courts that examples are not necessary in an application. The Board of Patent Appeals and Interferences stated in Ex parte Nardi and Simier, 229 USPQ 79 (BPA&I 1986):

The fact that the specification is devoid of working examples is without significance. It is well established that examples are not necessary. In re Borkowski, 57 CCPA 946, 422 F.2d 904, 164 USPQ 642.

This is supported by the Court of Appeals of the Federal Circuit in In re Strahilevitz, 212 USPQ 561, 563 (CAFC 1982). The Court stated:

We recognize that working examples are desirable in complex technologies and that detailed examples can satisfy the statutory enablement requirement. Indeed, the inclusion of such examples here might well have avoided a lengthy and no doubt, expensive appeal. Nevertheless, as acknowledged by the board, examples are not required to satisfy section 112, first paragraph. [Citations omitted].

In the present case, just to illustrate and demonstrate the usefulness of some of the claimed peptides, 18 examples have been presented. If each claimed peptide modification thereof, mixtures, conjugates and polymers thereof must be supported by an example with data, the burden on the inventor would be inordinate. The Applicant has provided ample proof that the immunodominant epitopes have been located and identified. From the clear teaching of the specification, it is clear that analogues of the claimed peptides can be constructed and that such analogues will be reactive to antibodies of HCV. Moreover, it is known that the HCV virus mutates and varying strains exist. If the analogues of the peptides must be specifically listed, the scope of the Applicant's claimed invention would be unfairly limited. The term analogue is clearly defined in the specification, and the method of making and using the analogues are clearly described. See, supra. This should be sufficient to satisfy 35 U.S.C. §112, first paragraph.

The Examiner also objected to the term "segments" in Claim 1. Applicant wish to point out that not only is the term "segments" of the peptides clearly defined, there are clear working examples showing the immunoreactivity of over forty segments of the claimed peptides.



See page 18 of the specification, which clearly defines what is meant by "segment" of a peptide; and Tables 1, 7 and 4 and Examples 1, 8 and 13. It is believed that the claimed invention directed to segments of the peptides is fully enabled.

The same can be said for the claim for mixtures of the peptides. Use of mixtures of the peptides are described in the specification in Examples 2, 4, 9, 10, 11, 12, 15, 16, 17 and 18. Therefore, the usefulness of mixtures of the peptides is also fully enabled.

Further, it is known to those of skill in the art that when each of the peptides are shown to be immunoreactive to HCV, a mixture of the peptides would necessarily also be immunoreactive to HCV. Therefore, undue experimentation is not required. For this reason, mixtures of the claimed peptides are enabled.

The Examiner also rejected conjugates and polymers of the peptides as not being enabled. The conjugates and polymers are clearly described on page 27, lines 17 to 22 and exemplified in Examples 3 and 7. Moreover, proteins which serve as conjugates to peptides and polypeptides such proteins are known to those of skill in the art. The synthesis of peptides attached to polymeric substrates are also known to those of skill in the art. The specific immunoreactivity to HCV of such conjugates and polymers is due to the peptide portion and not due to the conjugate or the polymeric part.

For the above reasons, it is believed that the invention as claimed is fully enabled and the rejection should be withdrawn.

The Examiner rejected Claims 1-11 and 28-34 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to point out and distinctly claim the subject matter of the invention.

Three reasons:

- (a) the term "analogues, segments, mixtures, combinations, conjugates and polymers thereof" fail to particularly point out and distinctly claim the peptides included in the claims.
- (b) the peptides of Claims 2-11 and 29-42 do not have antecedent basis in the generic claim,
- (c) Claim 43 is rejected as failing to define the other amino acids which can be present in the peptide.

Reconsideration of the rejection is requested.

As stated in the above, the specification and the originally filed claims clearly and distinctly defined the terms "analogues, segments, mixtures, combinations, conjugates and polymers" of the claimed peptides. Under the law, the claims are interpreted in view of the specification and the art. Not every detail of the invention need to be included in the claim.

In this case, the listing of every analogue, segment, mixture, conjugate and polymeric form of the claimed peptides would make the claims extremely unwieldy. Moreover, it would also unnecessarily limit the scope of the invention to which the Applicant is entitled. The present invention is broadly directed to the use of specific peptides as immunogens in the detection of antibodies to HCV for the diagnosis of the same. The Applicant is entitled to

every modification of these peptides including analogues, segments, mixtures, conjugates and polymeric forms of these peptides, which rely for their specific immunoreactivities on the fact that the Applicant has pointed to the peptides as described and claimed as the immunodominant regions of HCV. Each of the terms used in the claim is defined and supported by the specification. Therefore, it is believed that the claim as written clearly define the invention.

It is the Examiner's position that the peptides of claims 2-11 and 29-42 do not have antecedent basis in the generic claims.

It is believed that as amended, each of Claims 2-11 and 29-42 now has antecedent basis in Claim 1, the generic claim, and that the rejection on this basis has been overcome.

The Examiner rejected Claim 43 for failing the define the other amino acids which can be present in the peptide. As amended, Claim 43 clearly defines the remaining amino acids as corresponding to the amino acids sequence of HCV in the corresponding region of the HCV containing the epitope. It is believed that as amended, the rejection has been overcome.

The Examiner has cited but not applied the references listed in PTOL 892. No further comments are believed necessary.

It is believed that the claims as amended are allowable and an early allowance is requested.

Respectfully Submitted,

MORGAN & FINNEGAN

Dated: May 22, 1991 By: Maria C.H. Lin  
Maria C.H. Lin  
Registration No. 29,323

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## PATENT

Docket No. 1151-4043IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Chang Yi Wang                      Group Art Unit: 189  
Serial No. : 07/558,799                      Examiner: Lester Lee  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES FOR THE DETECTION OF ANTIBODIES TO HCV, DIAGNOSIS  
OF HCV INFECTION AND PREVENTION THEREOF AS VACCINES

AMENDMENT FEE TRANSMITTAL

HON. COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

Sir:

Transmitted herewith is an Amendment for the above-identified application.

☐ No additional fee is required.☒ The additional fee has been calculated as shown below:CLAIMS AS AMENDED

	Claims Remaining After Amendment	Highest No. Covered by Previous Payments	Present Extra	Rate	Additional Fee
Total Claims*	40	43	= 0	x \$20.00	\$ -0-
Independent Claims	18	5	= 13	x \$60.00	\$ 780.00
Multiple Dependent Claim(s)	(If claims added by amendment include Multiple Dependent Claim(s) and there was no Multiple Dependent Claim(s) in application before amendment add \$200.00 to additional fee.)				\$ -0-
Total:					\$ 780.00

☒ Verified Statement of "Small Entity" Status Under 37 CFR § 1.27  
filed July 26, 1990. Reduced Fees Under 37 CFR § 1.9(f)  
(50% of total) paid herewith

\$ 390.00

☐ Charge fee to Deposit Account No. 13-4500. Order No. \_\_\_\_\_  
A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

☒ The Commissioner is hereby authorized to charge any additional fees which may be required for this  
amendment, or credit any overpayment to Deposit Account No. 13-4500. Order No. 1151-4043.  
A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Includes all independent and single dependent claims and all claims referred to in multiple  
dependent claims. See 37 C.F.R. § 1.75(c).

Docket No. 1151-4043

- ☐        Page(s) of substitute Sequence Listing
- ☐        Computer disk(s) containing substitute Sequence Listing
- ☐ Statement under 37 C.F.R. § 1.825(b) that the computer and paper copies of the substitute Sequence Listing are the same.
- ☐ A check in the amount of \$            to cover the filing fee is attached.

Respectfully submitted,

MORGAN & FINNEGAN

Dated: May 22, 1991

By:

Maria C.H. Lin

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*18c 7-27-91*  
*Lee*  
PATENT  
U.S.S.N. 07/558,799

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Chang Yi Wang  
Serial No. : 07/558,799  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF  
ANTIBODIES TO HCV, DIAGNOSIS OF HCV INFECTION AND  
PREVENTION THEREOF AS VACCINES  
Group Art Unit : 189  
Examiner : Lester Lee

CERTIFICATE OF MAILING (37 C.F.R. 1.8a)

HON. COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

RECEIVED GROUP 18c  
JUN 03 1991

Sir:

I hereby certify that the attached AMENDMENT AND RESPONSE  
PURSUANT TO 35 C.F.R. §1.115 (along with any paper(s) referred to as being attached or  
enclosed) and this Certificate of Mailing are being deposited with the United States Postal  
Service on the date shown below with sufficient postage as first-class mail in an envelope  
addressed to the: Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Respectfully submitted,

MORGAN & FINNEGAN

Dated: May 22, 1991

By: *Maria C.H. Lin*

Maria C.H. Lin  
Registration No. 29,323

Mailing Address:

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UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	INVENTOR	ATTORNEY
07/558,799	07/26/90	WANG	LEE, L. FLEMMING

MORGAN & FINNEGAN  
345 PARK AVE.  
NEW YORK, NY 10154

LEE, L. FLEMMING

ATTORNEY  
189

08/15/91

This is a communication from the examiner in charge of your application.  
COMMISSIONER OF PATENTS AND TRADEMARKS

☐ This application has been examined ☒ Responsive to communication filed on May 28, 1991 ☒ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), \_\_\_\_\_ days from the date of this letter.  
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

- |   |  |
|---|--|
| 1. <input type="checkbox"/> Notice of References Cited by Examiner, PTO-892.        | 2. <input type="checkbox"/> Notice re Patent Drawing, PTO-948.                   |
| 3. <input type="checkbox"/> Notice of Art Cited by Applicant, PTO-1449.             | 4. <input type="checkbox"/> Notice of Informal Patent Application, Form PTO-152. |
| 5. <input type="checkbox"/> Information on How to Effect Drawing Changes, PTO-1474. | 6. <input type="checkbox"/> _____  |

Part II SUMMARY OF ACTION

1. ☒ Claims 1, 3, 5-6, 8-11, 28, 30-33 and 35-64 are pending in the application.

Of the above, claims \_\_\_\_\_ are withdrawn from consideration.

2. ☒ Claims 2, 4, 7, 12-27 and 29 and 34 have been cancelled.

3. ☒ Claims 44-46 and 48-54 are allowed.

4. ☒ Claims 1, 3, 5-6, 8-11, 28, 30-33, 35-43, 47 and 55-64 are rejected.

5. ☐ Claims \_\_\_\_\_ are objected to.

6. ☐ Claims \_\_\_\_\_ are subject to restriction or election requirement.

7. ☐ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.

8. ☐ Formal drawings are required in response to this Office action.

9. ☐ The corrected or substitute drawings have been received on \_\_\_\_\_. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable. ☐ not acceptable (see explanation or Notice re Patent Drawing, PTO-948).

10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on \_\_\_\_\_ has (have) been ☐ approved by the examiner. ☐ disapproved by the examiner (see explanation).

11. ☐ The proposed drawing correction, filed on \_\_\_\_\_, has been ☐ approved. ☐ disapproved (see explanation).

12. ☐ Acknowledgment is made of the claim for priority under U.S.C. 119. The certified copy has ☐ been received ☐ not been received  
☐ been filed in parent application, serial no. \_\_\_\_\_; filed on \_\_\_\_\_

13. ☐ Since this application appears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.

14. ☐ Other

EXAMINER'S ACTION



Serial No. 558799

-2-

Art Unit 189B

This action is responsive to the amendment filed May 28, 1991.

The following is a quotation of the first paragraph of 35 U.S.C. § 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

The specification is objected to under 35 U.S.C. § 112, first paragraph, as to adequately teach how to make and/or use the invention, i.e. failing providing an enabling disclosure.

The objection continues to be that as set forth in page 4 of paper No.4 with the exception that the term "combinations has been deleted.

Applicants' arguments have been considered but are not persuasive since there is nothing of record to show that the state of the art is advanced to the point where one skilled in the art can predict easily (without undue experimentation) the analogues, segments, mixtures, conjugates and polymers thereof which would be expected to detect antibodies to hepatitis C virus.

Claims 1,3,5-6,8-11,28,30-33 and 35-43 are rejected under 35 U.S.C. § 112, first paragraph, for the reasons set forth in the objection to the specification.

Serial No. 558799

-3-

Art Unit 189B

Claims 1,3,5-6,8-11,28,30-33 and 35-43 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The claims continue to be rejected for the reasons set forth on page 4 (a) of paper No.4.

Applicant's arguments have been considered but are not persuasive since they do not establish how the recited terms limit the claimed peptides to specific analogues, segments, mixtures, conjugates and polymers thereof.

Claims 44-46 and 48-54 are allowable.

Claims 47 and 55-64 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

(a) Claim 47 is indefinite since the amino acid sequence is wrong- LysGlnLys,

(b) Claims 55-64 are indefinite since the claims do not list the peptides that I, IIF, II, III, IIID, IV, V, VI, VII, VIII and IXD represent.

Applicant's amendment necessitated the new grounds of rejection. Accordingly, THIS ACTION IS MADE FINAL. See M.P.E.P. § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 C.F.R. § 1.136(a).

Serial No. 558799

-4-

Art Unit 189B

A SHORTENED STATUTORY PERIOD FOR RESPONSE TO THIS FINAL ACTION IS SET TO EXPIRE THREE MONTHS FROM THE DATE OF THIS ACTION. IN THE EVENT A FIRST RESPONSE IS FILED WITHIN TWO MONTHS OF THE MAILING DATE OF THIS FINAL ACTION AND THE ADVISORY ACTION IS NOT MAILED UNTIL AFTER THE END OF THE THREE-MONTH SHORTENED STATUTORY PERIOD, THEN THE SHORTENED STATUTORY PERIOD WILL EXPIRE ON THE DATE THE ADVISORY ACTION IS MAILED, AND ANY EXTENSION FEE PURSUANT TO 37 C.F.R. § 1.136(a) WILL BE CALCULATED FROM THE MAILING DATE OF THE ADVISORY ACTION. IN NO EVENT WILL THE STATUTORY PERIOD FOR RESPONSE EXPIRE LATER THAN SIX MONTHS FROM THE DATE OF THIS FINAL ACTION.

Any inquiry concerning this communication should be directed to Lester L. Lee at telephone number (703) 308-3997.

Lee/sg  
August 05, 1991

*Lester L. Lee*  
LESTER L. LEE  
PRIMARY PATENT EXAMINER  
ART UNIT 189B  
1275



1151-4043

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PATENT  
U.S.S.N. 07/558,799

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Chang Yi Wang  
Serial No. : 07/558,799  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE  
DETECTION OF ANTIBODIES TO HCV,  
DIAGNOSIS OF HCV INFECTION AND  
PREVENTION THEREOF AS VACCINES  
Group Art Unit : 189  
Examiner : Lester Lee

Hon. Commissioner of Patents and Trademarks  
Washington, D.C. 20231

AMENDMENT AND RESPONSE PURSUANT to 35 C.F.R. 1.116  
AFTER FINAL REJECTION

Sir:

This is submitted in response to the Office Action  
dated August 15, 1991 for which a three month response  
period was set.

Enclosed herewith is a set of formal drawings  
Figs. 1-15 (27 sheets) to replace the informal drawings  
filed with the application.

AMENDMENT

Please amend claims 1, 3, 5, 6, 8, 9, 30-33, 35-42,  
47, and 55, 59-61 as follows:

*Clan*  
*10/27/91*

*9/20/91*  
*And to C*  
*(HE)*

*Informal*  
*Drawings*

*Don't*  
*10/27/91*  
*8.20*  
*10/16/91*  
*K*  
*10/27/91*  
*2.20*  
*11/15/91*  
*B.W. White*  
*11-22-91*  
*10/27/91*  
*8.20*

1 (Twice Amended). A peptide composition comprising a peptide selected from the group consisting of Peptide I to IX each peptide with an amino acid sequence as follows:

- Sub D1*
- (i) Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X  
(Peptide I)
- (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X  
(Peptide II)
- (iii) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X  
(Peptide III)
- (iv) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X  
(Peptide IV)
- (v) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X  
(Peptide V)
- (vi) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X  
(Peptide VI)
- (vii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X  
(Peptide VII)

(viii) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-  
Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-  
Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-  
Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-  
Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-  
Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-  
Arg-X

(Peptide VIII)

and

(ix) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-  
Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-Gln-Pro-  
Gly-Tyr-Pro-Trp-Pro-Leu-Thr-Gly-Asn-Glu-  
Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-  
Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-  
Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-  
Gly-X

(Peptide IX)

wherein X is -OH or -NH<sub>2</sub>; and

- (x)
- a. an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%;
  - b. a segment of each of the above peptides or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%;
  - c. a mixture of the above peptides or analogues of the peptides;
  - d. a conjugate of each of the peptides with carrier proteins, the conjugate having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%;
- and

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a. a polymer of each of the peptides  
comprising a branching dimer, tetramer, or  
octomer of the peptide on a mono, tri, or  
hepta lysine core respectively.

[analogues, segments, mixtures, conjugates  
and polymers thereof.]

12  
C2

3 (Twice Amended). A peptide composition  
according to Claim 1 wherein the peptide comprises a segment  
of Peptide II and has an amino acid sequence selected from  
the group consisting of:

- (i) Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-  
Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-  
Leu-Gly-Leu-X;  
(Peptide IIC)
- (ii) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-  
Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-  
Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IID)
- (iii) Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-  
Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-  
Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-  
Gly-Leu-X;  
(Peptide IIE)
- (iv) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-  
Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-  
Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IIF)

wherein X is -OH or -NH<sub>2</sub> and [analogues thereof.]

an analogue of each of the above peptides having  
an amino acid sequence derived from a  
strain/isolate of HCV in a region corresponding to  
the peptide and having specific immunoreactivity  
to antibodies to HCV relative to the peptide of at  
least 20%.

D3  
C3

5 (Twice Amended). A peptide composition according to Claim 1 wherein the peptide comprises a segment of Peptide III and has an amino acid sequence selected from the group consisting of:

- (i) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;  
(Peptide IIIC)
- (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;  
(Peptide IIID)
- (iii) Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;  
(Peptide IIIE)

wherein X is - OH or -NH<sub>2</sub> and [analogues thereof.]

an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%.

6 (Twice Amended). A peptide composition according to Claim 5 wherein the peptide has an amino acid sequence as follows:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

(Peptide IIID)

wherein X is -OH or -NH<sub>2</sub> or an [analogue thereof.]

an analogue of the above peptide having an amino acid sequence derived from a strain/isolate of HCV



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in a region corresponding to the peptide and  
having specific immunoreactivity to antibodies to  
HCV relative to the peptide of at least 20%.

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C4

8 (Twice Amended). A peptide composition according to Claim 1 wherein the peptide comprises a segment of Peptide VIII and has an amino acid sequence selected from the group consisting of:

- (i) Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIIID)
- (ii) Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIIIC)
- (iii) Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIIIB)
- (iv) Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIIIA)

wherein X is -OH or -NH<sub>2</sub> or an [analogue thereof.]

analogue of each of the above peptides having an  
amino acid sequence derived from a strain/isolate  
of HCV in a region corresponding to the peptide  
and having specific immunoreactivity to antibodies  
to HCV relative to the peptide of at least 20%.

9 (Twice Amended). A peptide composition according to Claim 1 wherein the peptide comprises a segment of Peptide IX and has an amino acid sequence selected from the group consisting of:

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- (i) Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;  
(Peptide IXD)
- (ii) Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;  
(Peptide IXC)
- (iii) Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;  
(Peptide IXB)
- (iv) Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;  
(Peptide IXA)

wherein X is -OH or -NH<sub>2</sub> or an [analogue thereof.]

analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%.

10. A peptide composition according to Claim 1 wherein the peptide has an amino acid sequence as follows:

Ser-Thr-Pro-Lys-Pro-Gln-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X

wherein X is -OH or -NH<sub>2</sub>, or an analogue [thereof].

of the above peptide having an amino acid sequence  
derived from a strain/isolate of HCV in a region  
corresponding to the peptide, and having specific  
immunoreactivity to antibodies to HCV relative to  
the peptide of at least 20%; and

a segment of the above peptide having specific  
immunoreactivity to antibodies to HCV relative to  
the peptide of at least 20%.

11. A peptide composition according to Claim 1

wherein the peptide has an amino acid sequence as follows:

Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-  
Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-  
Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-  
Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-  
Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-  
Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-  
Gly-X;

wherein X is -OH or -NH<sub>2</sub>, an analogue[s] [thereof].

of the above peptide having an amino acid sequence  
derived from a strain/isolate of HCV in a region  
corresponding to the peptide, and having specific  
immunoreactivity to antibodies to HCV relative to  
the peptide of at least 20%; and

a segment of the above peptide or analogue thereof  
having specific immunoreactivity to antibodies to  
HCV relative to the peptide of at least 20%.

30 (Amended). An ELISA test kit according to claim 28 wherein the [multiple wells are] solid phase is coated with a peptide composition comprising a segment of Peptide II and having an amino acid sequence selected from the group consisting of:

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- (i) Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
  - (ii) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
  - (iii) Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
  - (iv) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
  - (v) [Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
  - (vi)] Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

wherein X is -OH or -NH<sub>2</sub>, and [analogues thereof.]

an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%.

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31 (Amended). An ELISA test kit according to claim  
28 wherein [the multiple wells are] solid phase is coated  
with a peptide having an amino acid sequence:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

wherein X is OH or -NH<sub>2</sub> and [analogues thereof.]

an analogue of the above peptide having an amino  
acid sequence derived from a strain/isolate of HCV  
in a region corresponding to the peptide and having  
specific immunoreactivity to antibodies to HCV  
relative to the peptide of at least 20%.

32 (Amended). An ELISA test kit according to claim  
28 wherein the [multiple wells are] solid phase is coated  
with a peptide composition comprising a segment of Peptide  
III having an amino acid sequence selected from the group  
consisting of:

- (i) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-  
Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-  
Pro-Tyr-Ile-X;
- (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-  
Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-  
Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;
- (iii) Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-  
Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-  
Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-  
Tyr-Ile-X;
- ((iv) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-  
Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-  
Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-  
Ser-Gln-His-Leu-Pro-Tyr-Ile-X;]

wherein X is -OH or -NH<sub>2</sub> and [analogues thereof.]

an analogue of each of the above peptides having an  
amino acid sequence derived from a strain/isolate

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of HCV in a region corresponding to the peptide and  
having specific immunoreactivity to antibodies to  
HCV relative to the peptide of at least 20%.

33 (Amended). An ELISA test kit according to claim 32 wherein the peptide is:

- (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

wherein X is -OH or -NH<sub>2</sub> or an [analogue thereof.]

analogue of the above peptides having an amino acid  
sequence derived from a strain/isolate of HCV in a  
region corresponding to the peptide and having  
specific immunoreactivity to antibodies to HCV  
relative to the peptide of at least 20%.

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35 (Amended). An ELISA test kit according to Claim 28 wherein [the multiple wells are] solid phase is coated with a peptide composition comprising a segment of Peptide VIII having an amino acid sequence selected from the group consisting of:

- (i) Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;
- (ii) Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;
- (iii) Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;
- (iv) Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;

wherein X is -OH or -NH<sub>2</sub> or an [analogue thereof.]

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cont

analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%.

36. An ELISA test kit according to Claim 28 wherein [the multiple wells are] solid phase is coated with a peptide composition comprising a segment of Peptide IX having an amino acid sequence selected from the group consisting of:

- (i) Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (ii) Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (iii) Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (iv) Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue [thereof.]

of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%.

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37 (Amended). An ELISA test kit according to Claim 28 wherein the [multiple wells are] solid phase is coated with a peptide composition comprising a peptide having an amino acid sequence as follows:

Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-  
Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-  
Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-  
Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-  
Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-  
Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-  
Arg-X

wherein X is -OH or -NH<sub>2</sub> or an [analogue thereof.]

analogue of the above peptide having an amino acid  
sequence derived from a strain/isolate of HCV in a  
region corresponding to the peptide, and having  
specific immunoreactivity to antibodies to HCV  
relative to the peptide of at least 20%; and  
a segment of the above peptide or analogue thereof  
having specific immunoreactivity to antibodies to  
HCV relative to the peptide of at least 20%.

38 (Amended). An ELISA test kit according to Claim 28 wherein the [multiple wells are] solid phase is coated with a peptide composition comprising a peptide having an amino acid sequence as follows:

Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-  
Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-  
Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-  
Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-  
Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-  
Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-  
Gly-X;

wherein X is -OH or -NH<sub>2</sub> and [analogues thereof.]



an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%.

39 (Amended). An ELISA test kit according to Claim 28 wherein the [multiple wells are] solid phase is coated with a peptide composition comprising a mixture of Peptides IIH and V, Peptides IIH and V having the following amino acid sequences respectively:

- (i) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X (IIH)
- (ii) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X; (V)

wherein X is -OH or -NH<sub>2</sub> or [analogues thereof.]

an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%.

40 (Amended). An ELISA test kit according to Claim 28 wherein the [multiple wells are] solid phase is coated with a peptide composition comprising a mixture of Peptides IIF, IIID and V, Peptide IIF, IIID and V having the following amino acid sequences respectively:

- (i) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X; (IIF)  
 (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X; (IIID)  
 (iii) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X; (V)

wherein X is -OH or -NH<sub>2</sub> or [analogues thereof.]

an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%.

41 (Amended). An ELISA test kit according to Claim 28 wherein the [multiple wells are] solid phase is coated with a peptide composition comprising a mixture of Peptides IIH, V and VIIIE, Peptide IIH, V and VIIIE having the following amino acid sequences respectively:

- (i) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X; (IIH)  
 (ii) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X; (V)  
 (iii) Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X; (VIIIE)

wherein X is -OH or -NH<sub>2</sub> or an [analogue thereof.]

an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%.

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42 (Amended). An ELISA test kit according to Claim 28 wherein the [multiple wells are] solid phase is coated with a peptide composition comprising a mixture of Peptides VIIIE and IXD, Peptide VIIIE and IXD having the following amino acid sequences respectively:

- (i) Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-X; (VIIIE)
- (ii) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X; (IXD)

wherein X is -OH or -NH<sub>2</sub> and [analogues thereof.]

an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide, and having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%; a segment of each of the above peptide or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 20%.

C7 47<sup>34</sup> (Amended). A peptide having the amino acid sequence:

Lys-Gl[y]<sub>n</sub>-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X

(Peptide V)

wherein X is -OH or NH<sub>2</sub>.

C8 58<sup>34</sup> (Amended). An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

(i) a solid [substrate] phase coated with a peptide composition containing any one of the following peptides:

Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X;

(Peptide I)

Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

(Peptide IIF)

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

(Peptide II)

Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

(Peptide III)

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

(Peptide IIID)

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X;

(Peptide IV)

Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X;

(Peptide V)

Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X;

(Peptide VI)

Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X;

(Peptide VII)

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X; and

(Peptide VIII)

Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

(Peptide IXD)

[I, IIF, II, III, IIID, IV, V, VI, VII, VIII, and IXD] or mixtures thereof;

- (ii) a negative control sample; and
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG;
- and
- (vi) an enzyme substrate.

Sub G3  
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59 (Amended). An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

(i) wherein the peptide composition comprises a mixture of

C9  
Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X; and  
(Peptide II)

Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X

(Peptide V)

[Peptides II and V];

- (ii) a negative control sample; and
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.

34  
60 (Amended). An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

(i) wherein the peptide composition comprises a mixture of

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X; and  
(Peptide II)

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X

(Peptide VIII)

[Peptides II and VIII];

- (ii) a negative control sample; and
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.

31 (Amended). An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

- (i) wherein the peptide composition comprises a mixture of

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

(Peptide II)

Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X; and

(Peptide V)

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X

(Peptide VIII)

[Peptides II, V and VIII];

- (ii) a negative control sample; and

*C9  
concluded*

(iii) an inactivated HCV positive control  
sample;

(iv) specimen diluent;

(v) enzyme labelled antibodies to human IgG;

and

(vi) an enzyme substrate.

Please cancel Claims 43, 56, 57 and 58 without  
prejudice.



REMARKS

Claims 1-9 have been amended to more clearly define analogues, segments, mixtures, conjugates and polymers of the peptides. Support for the amendment can be found in the specification for:

- I. Analogues - Page 6, lines 26 - Page 9, line 12; and Page 27, lines 5-16;
- II. Segments - Page 18, lines 16-18; Table 1, Page 31; Table 7, Page 32; Table 2, Page 40; Table 5, Page 67 originally filed claims 3, 5, 8 and 9;
- III. Mixtures - Examples 2, 8, 9, 10, 12, 15, 16, 17 and 18;
- IV. Conjugates - Page 27, lines 17-22 and Example 3; and
- V. Polymers - Page 27, lines 19-22 and Example 7.

No new matter has been introduced. Entry of the amendment is requested.

A set of formal drawings are submitted herewith in accordance with a telephone discussion with the Examiner. The drawings have been prepared based on the informal drawings filed with the present application. The descriptive matter in each figure has been removed to conform to rules of 37 CFR 1.81(g). No new matter has been introduced on the drawings. Entry of the drawings is requested.

The Examiner has indicated that claims 44-46 and 48-54 are allowable, claim 47 is allowable if amended to correct the amino acid sequence and claims 55-64 is allowable if amended to recite the specified peptides.

Claims 47 and 55-64 have been amended as suggested and the rejection of these claims under 35 U.S.C. §112, second paragraph as being indefinite has been overcome.

The Examiner has rejected claims 1, 3, 5-6, 8-11, 28, 30-33 and 35-43 under 35 U.S.C. §112 first paragraph, for failing to provide an enabling disclosure.

An interview was had with the Examiner, who explained that the terms "analogues, segments, mixtures, conjugates and polymers" are overly broad. If these terms are narrowed by recitation of their specific immunoreactivity to antibodies to HCV with a specified relative immunoreactivity to the peptide, the rejection would be overcome.

These claims have been narrowed to recite that the analogues and segments have specific immunoreactivity to HCV relative to the parent peptide of at least 20%, and the terms "mixtures, conjugates and polymers" have been now specifically defined. Support for this amendment is also shown in the specification.

To further demonstrate that the analogues are effective and have specific immunoreactivities to antibodies to HCV, enclosed herewith is the Declaration by Dr. Chang Yi Wang, the inventor. The three experiments illustrate the effectiveness of the analogues which were described on Page 27, lines 5-16 of the specification.

As amended, Claims 1, 3, 5-6, 8-11, 30-33, and 35-42 are allowable.

The Examiner's courtesy and helpful suggestions during the interview is deeply appreciated.

The claimed invention has proven to be commercially successful. Diagnostic test kits containing the claimed peptides have been approved and sold in Europe. Based on sales for the past few months, it has captured about 20% of the market in certain European countries for screening HCV in blood supplies. Approval from FDA is expected.

It is the Applicant's position that the claims as amended are allowable and an early allowance is requested.

Respectfully Submitted,

MORGAN & FINNEGAN

Dated: September 25, 1991

By: Maria C.H. Lin  
Maria C.H. Lin  
Registration No. 29,323

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(212) 751-6849 Telecopier



PATENT  
U.S.S.N. 07/558,799

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Chang Yi Wang  
Serial No. : 07/558,799  
Filed : July 26, 1991  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE  
DETECTION OF ANTIBODIES TO HCV, DIAGNOSIS  
OF HCV INFECTION AND PREVENTION THEREOF  
AS VACCINES  
Group Art Unit : 189  
Examiner : L. Lee

Hon. Commissioner of Patents and Trademarks  
Washington, D.C. 20231

DECLARATION OF DR. CHANG YI WANG

I, Chang Yi Wang, declare:

1. I am the named inventor of the above identified application.
2. I received a Ph.D. degree in Immunology and Biochemistry  
from The Rockefeller University in 1979.
3. I conceived the idea of using synthetic peptides as  
immunoreactive reagents specific for the detection of the presence of antibodies to  
hepatitis C virus (HCV) in body fluids for the diagnosis of non-A, non-B hepatitis  
(NANBH).
4. My conception includes the use of analogues of the synthetic  
peptide.
5. My conception is based on my knowledge that when an  
immunoreactive peptide for a particular virus has been identified through an extensive

6. The following analogue peptides were synthesized and tested in my laboratory under my direction and control.

Analogue peptides with the following amino acid (aa) sequences were synthesized using the classical Merrifield method of solid phase peptide synthesis. The aa sequences are based on the amino acid sequence of the envelope region of two strains of HCV, the PT and J4 strains.

The (:)s show where amino acid substitutions have been made. In this case six substitutions (15%) were made.

The absorbance at 492 nm ( $OD_{492nm}$ ) was measured.

Table I

<u>Serum Code</u>	<u>OD<sub>492nm</sub></u>	
	<u>254B</u>	<u>322</u>
NABI 2-6	0.283	0.294
NABI 26	0.396	0.432
NABI 33	0.650	<u>1.139</u>
NABI 38	<u>1.915</u>	0.472
NABI 41	0.502	0.527
NABI 44	0.800	<u>1.252</u>
UBI #6	1.550	<u>2.219</u>
NABI 2-48	3.599	3.475
% Relative Immunoreactivity:		
	9.695	9.810
	100	101.2

The specific immunoreactivity to HCV of the analogue relative to Peptide 254 was 101.2%.

Example II

Another pair of analogues were prepared and tested by ELISA according to the procedures described in the specification of the present application. These analogues contained aa sequences corresponding to the NS-4 region of HCV as follows:

Peptide 204 H (IIIH)  
 SGKPA, IIPDR, EVLYR, EFDEM, EEC SQ, HLPYI, EGQMM, LAEQF, KQKAL, GL  
 : : : : :  
 Peptide 3K204H'  
 KKK, SGRPA, VVPDR, ELLYQ, EFDEM, EECAS, HLPYI, EGQMQ, LAEQF, KQKAL, GL

There are eight aa (17%) substitutions and three lysine additions at the N-terminus.

Using eight HCV Positive serum samples from patients ELISA assays using Peptide IIH and its analogue Peptide 3K204H' as solid phase immunoabsorbents, the OD<sub>492nm</sub> were shown as follows:

TABLE II

<u>Serum Code</u>	<u>OD<sub>492nm</sub></u>	
	<u>204H</u>	<u>KKK-204H'</u>
UBI #1	2.242	2.859
UBI #2	0.574	0.392
UBI #3	3.250	3.074
UBI #4	2.867	2.713
UBI #5	0.739	0.186
UBI #6	2.003	1.683
UBI #7	2.235	2.211
UBI #8	3.493	3.494
BG	0.022	0.024
NRC	0.068	0.056
% Relative Immunoreactivity:		
	17.403	16.612
	100	95.5

The results showed the specific immunoreactivity to HCV of the analogue to HCV relative to Peptide IIH is 95.5%.

### Example III

An analogue of Pep11 was synthesized and tested by ELISA according to the procedures described in the specification of the present application. The aa sequence of the analogue Peptide 309C' compared with Pep11 is as follows:

Peptide 309C(Peptide 11):

ARPDY, NPPLV, ETWKK, PDYEP, PVVHG, CPLPP, PKSPP, VPPPR, KKRT  
: : : :

Peptide 309C'

ARPDY, NPPLI, ESWKD, PDYEP, PVVHG, CPLPP, PKAPP, VPPPR, KKRT

There are, therefore, four aa (~10%) substitutions in the sequence. The ELISA results for eight HCV positive serum samples are shown as follows:

TABLE III

<u>Serum Code</u>	<u>OD<sub>492nm</sub></u>	
	<u>309C</u>	<u>309C'</u>
UBI #1	0.323	0.050
UBI #2	0.024	0.072
UBI #3	3.194	3.163
UBI #4	1.054	1.679
UBI #5	0.576	0.540
UBI #6	0.959	1.325
UBI #7	0.490	0.933
UBI #8	3.796	3.493



<u>Serum Code</u>	<u>309C</u>	<u>309C'</u>
BG	0.012	0.002
NRC	0.017	0.026
% Relative Immunoreactivity:		
	10.416	11.255
	100	108

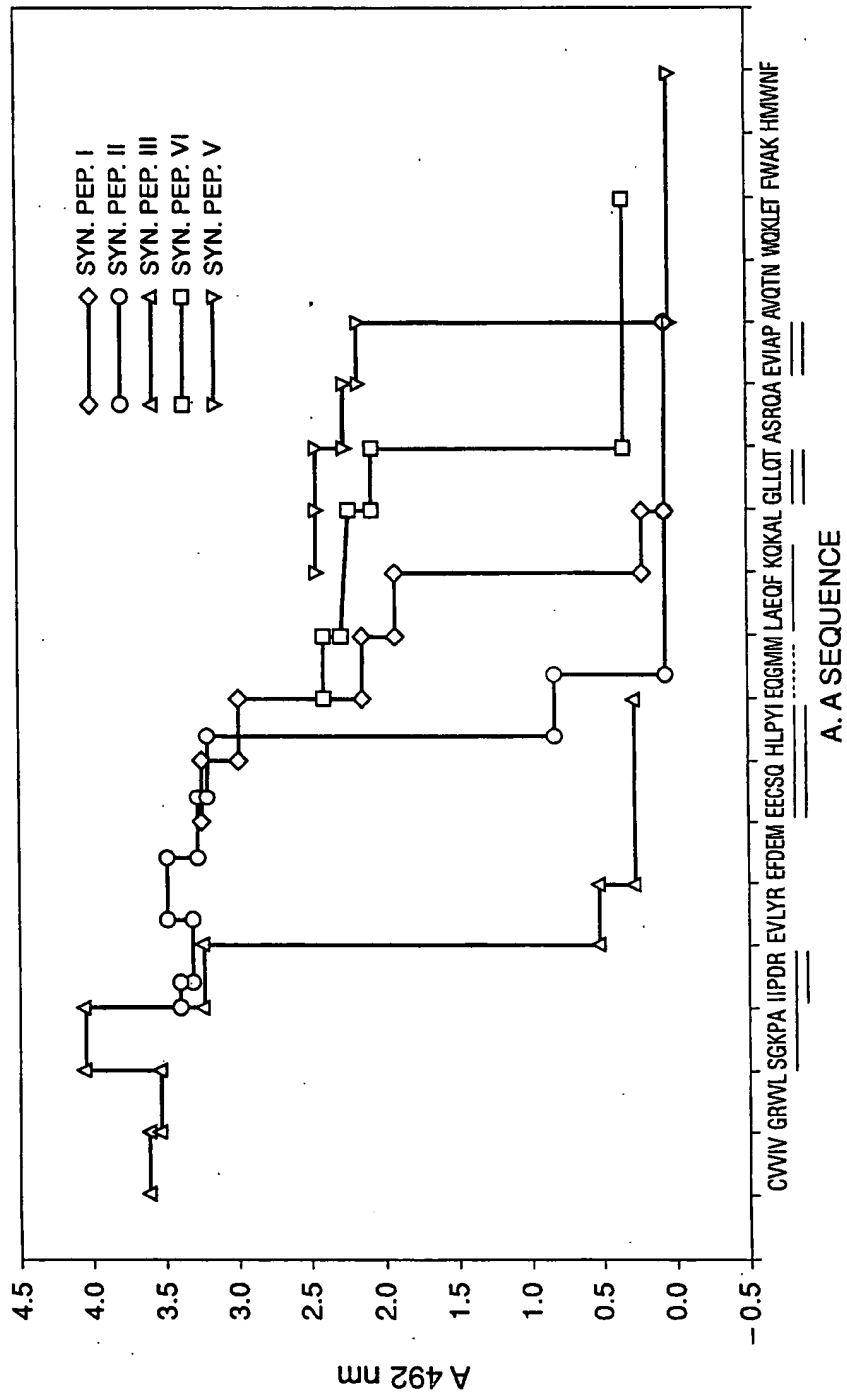
The specific immunoreactivity to HCV of the analogue relative to Pep11 was 108%.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are found to be true, and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of The United States Code, and that such wilful, false statements may jeopardize the validity of the application, any patent issuing thereon.

Date:

September 17, 1991 Dr. Chang Yi Wang

FIG. 1-1.



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None

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FIG. 1-2.

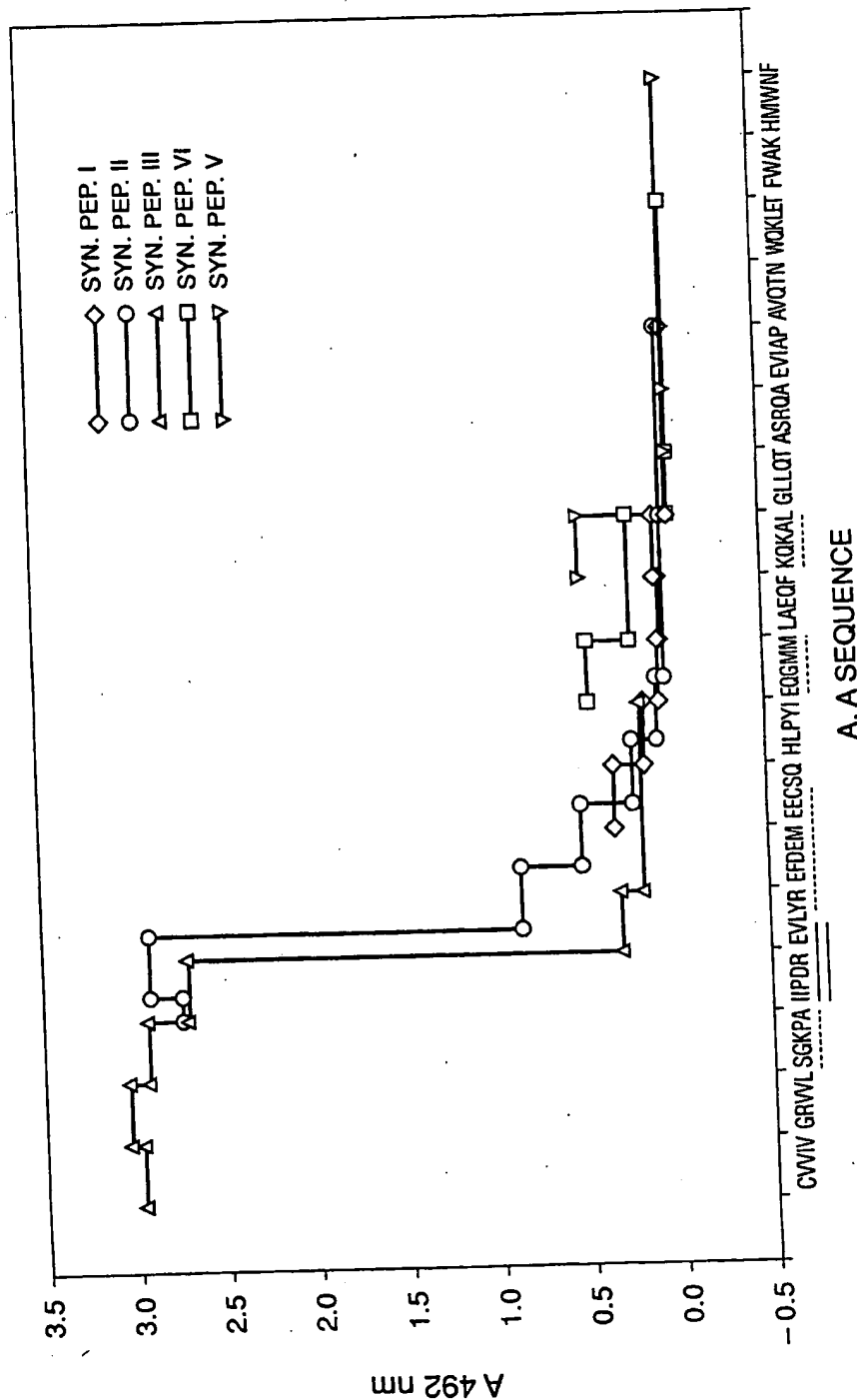
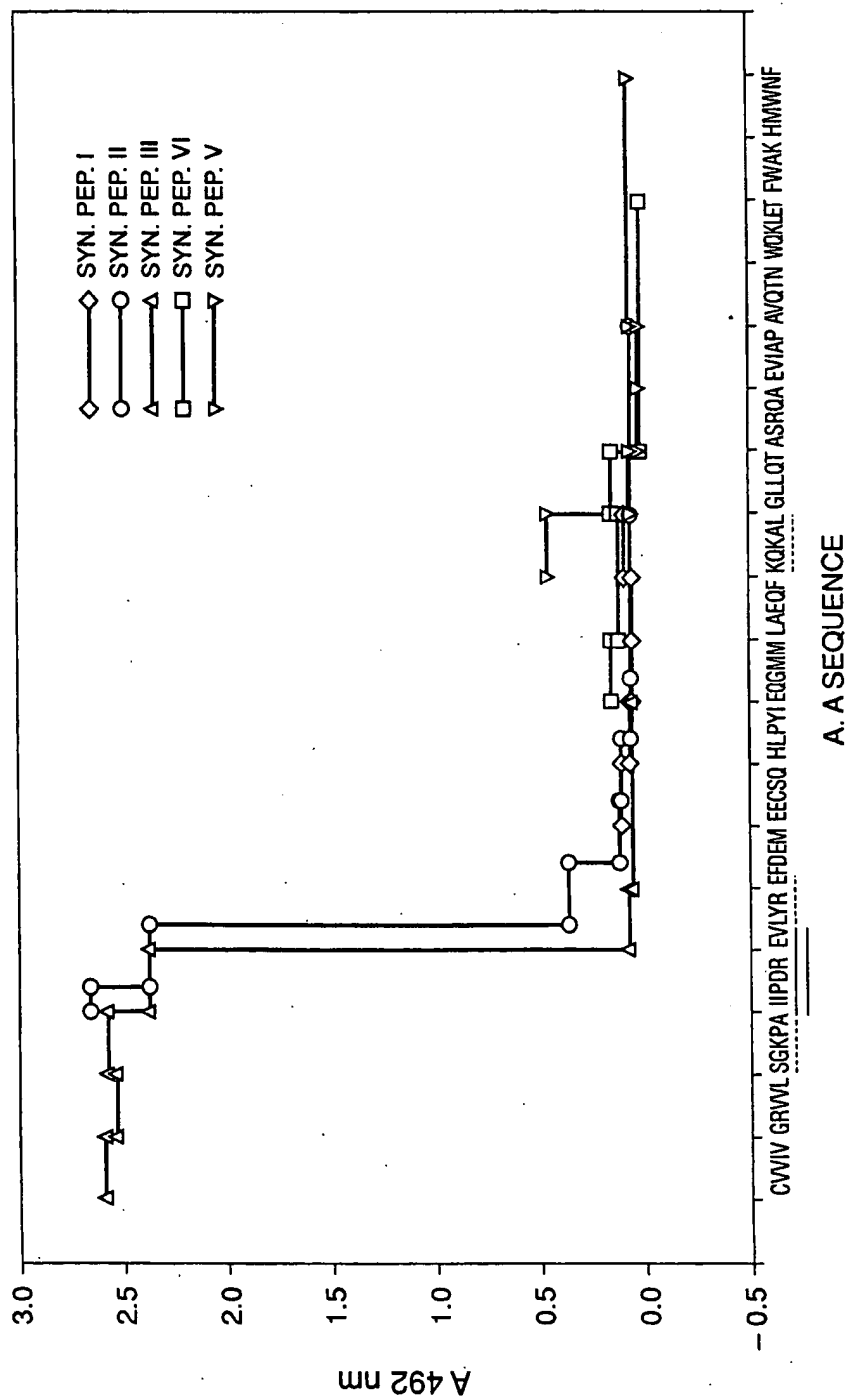


FIG. 1-3.

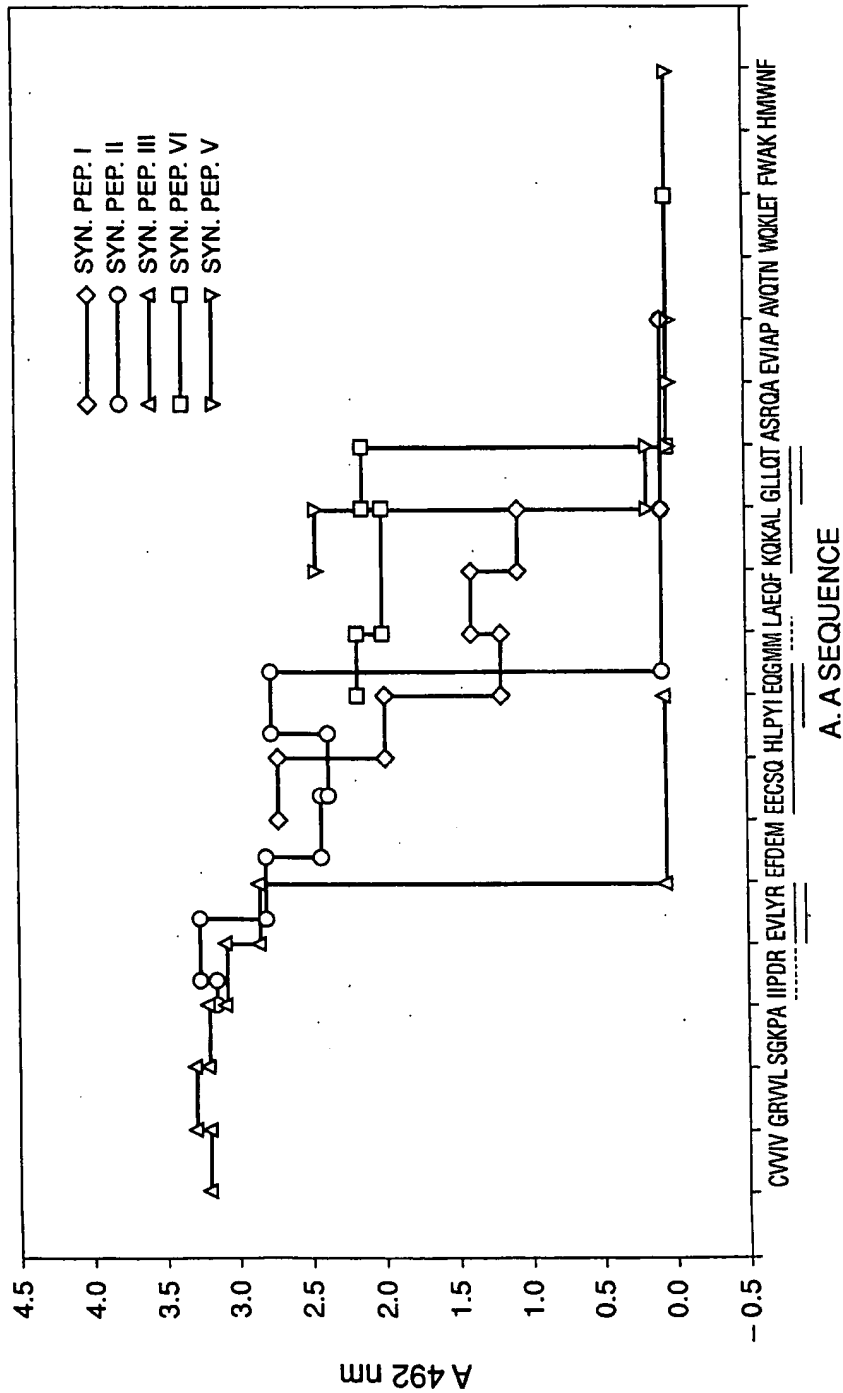


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FIG. 1-4.



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None

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None

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FIG. 2-1.

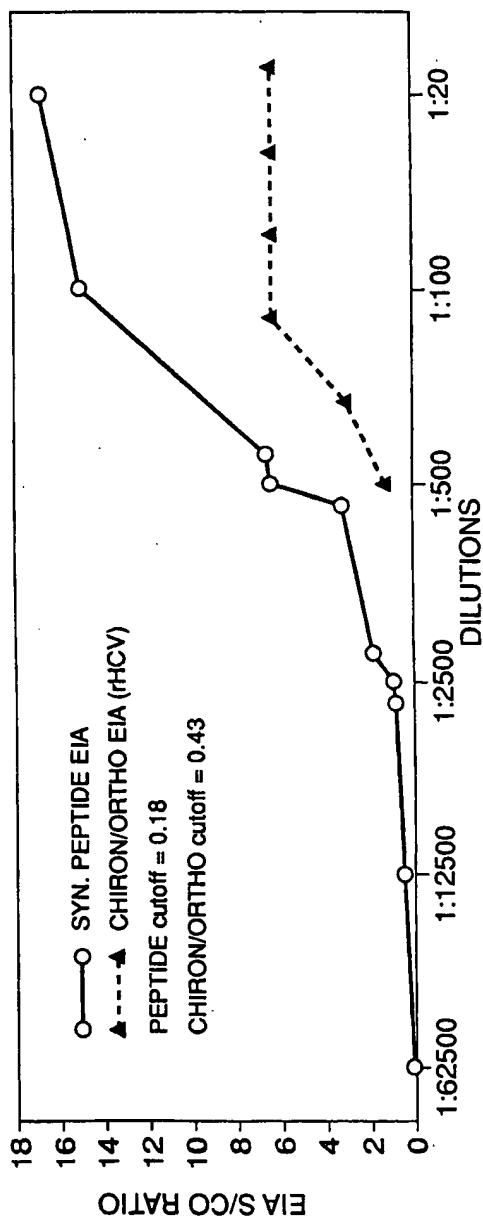
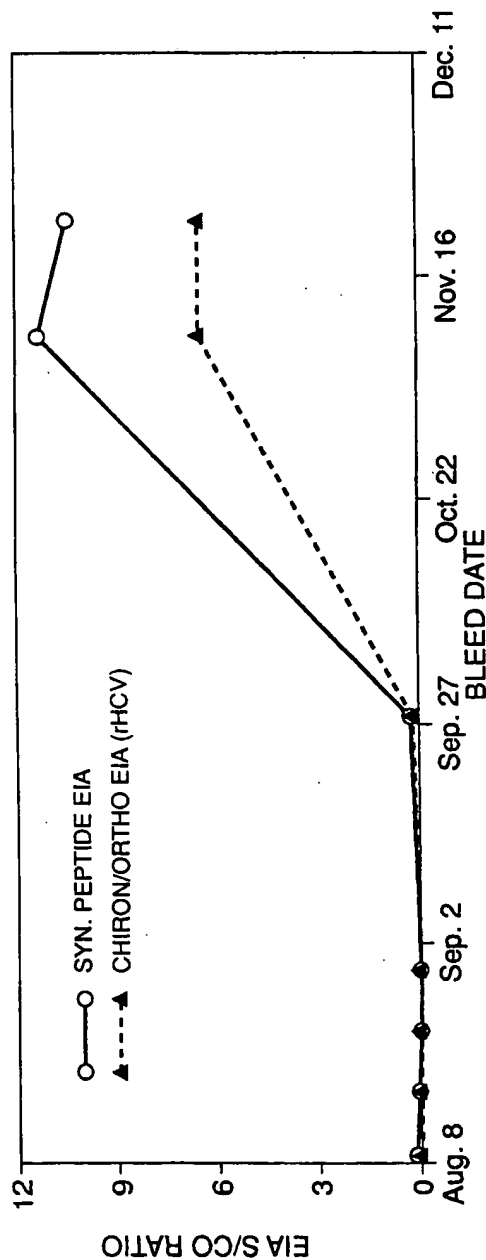


FIG. 2-2.



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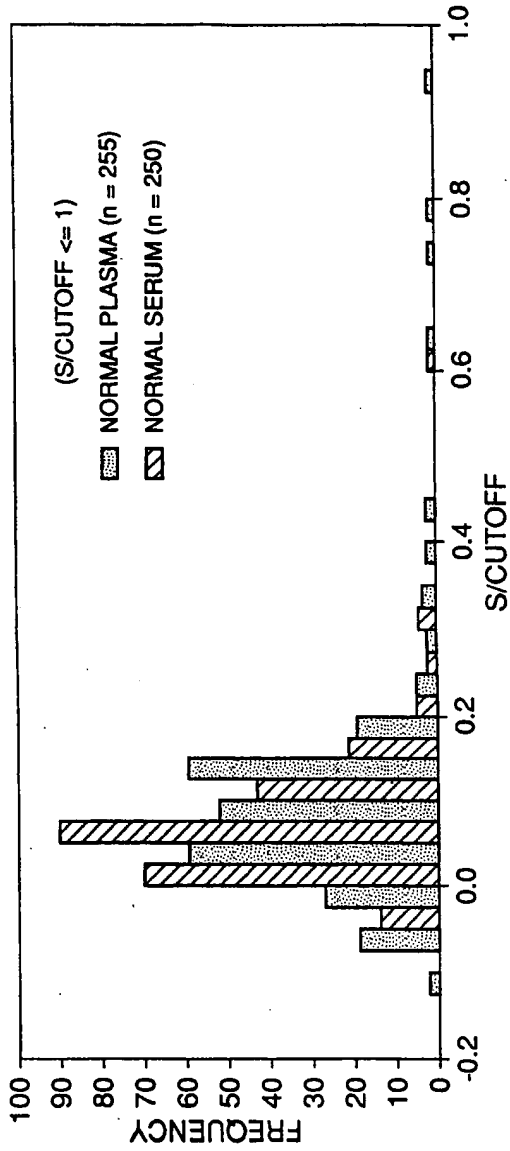


FIG. 3-1.

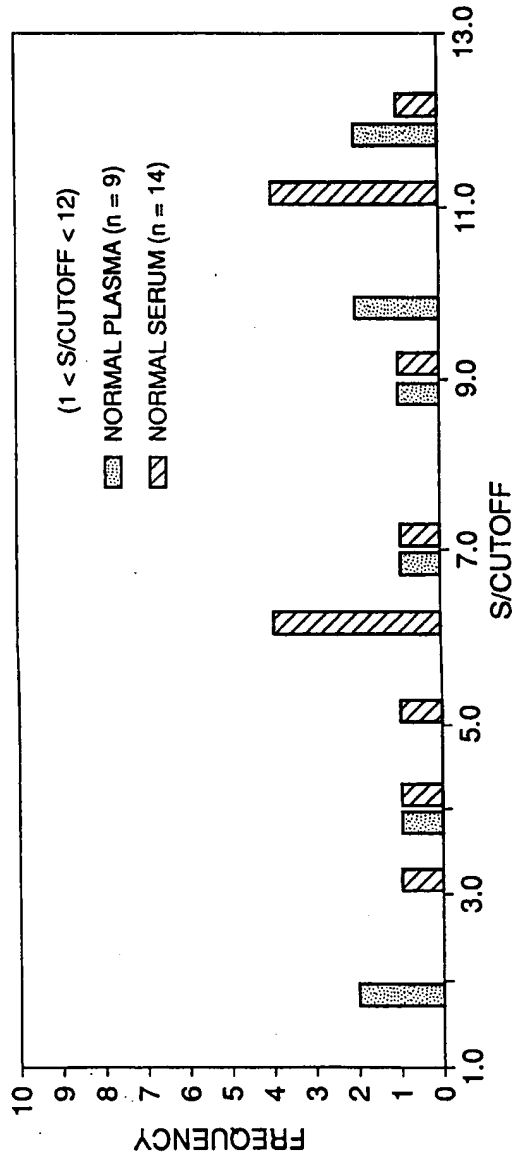
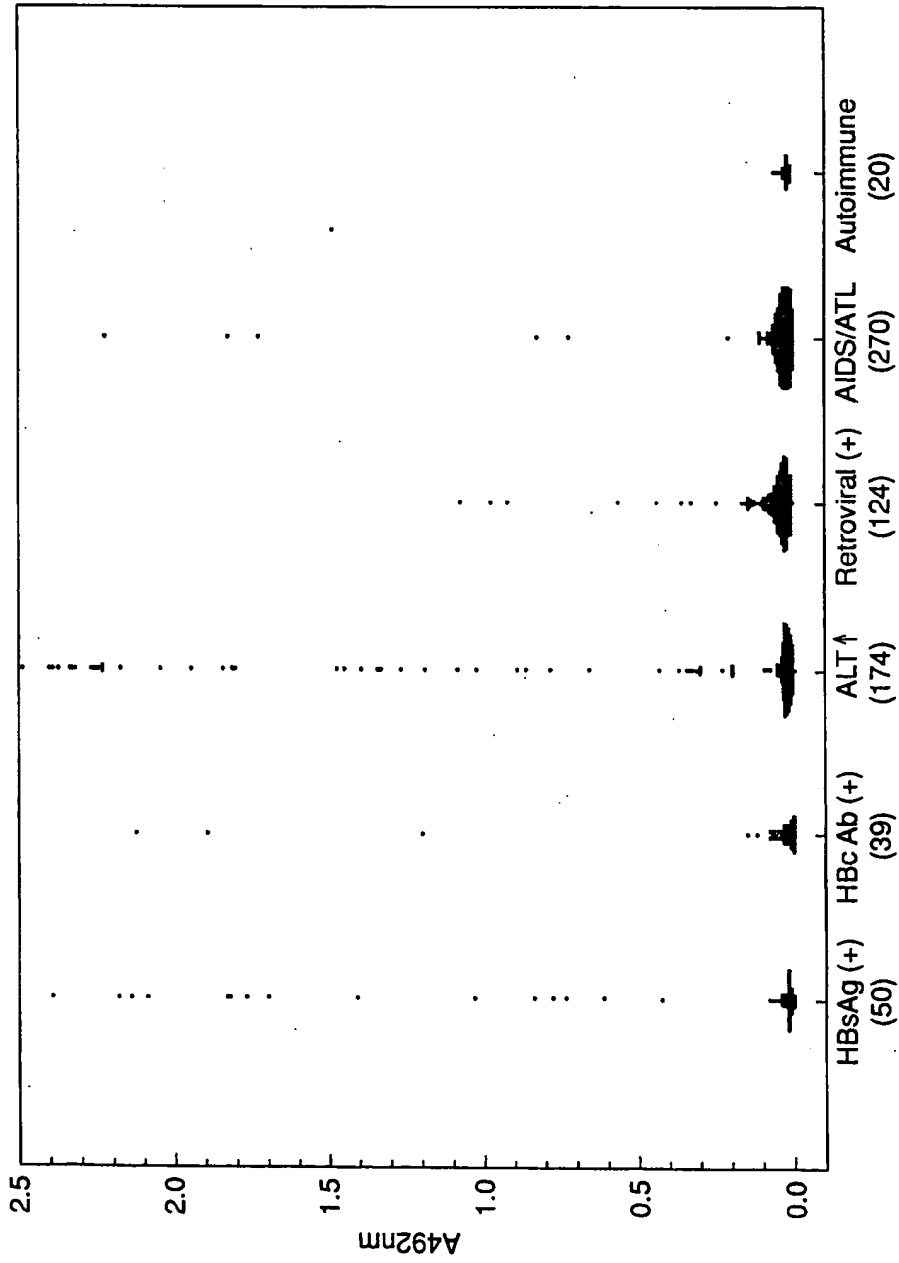


FIG. 3-2.

FIG. 4.



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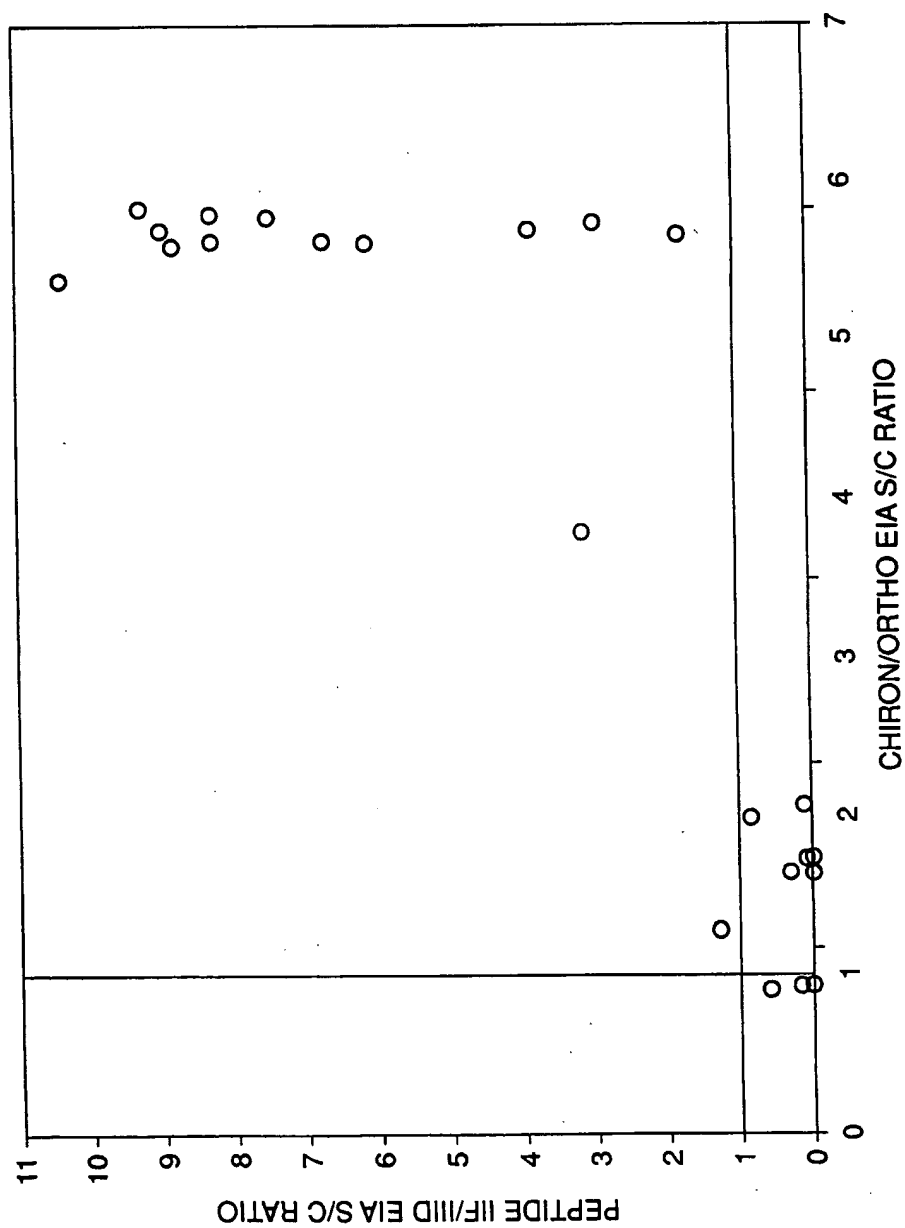
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none

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FIG. 5.

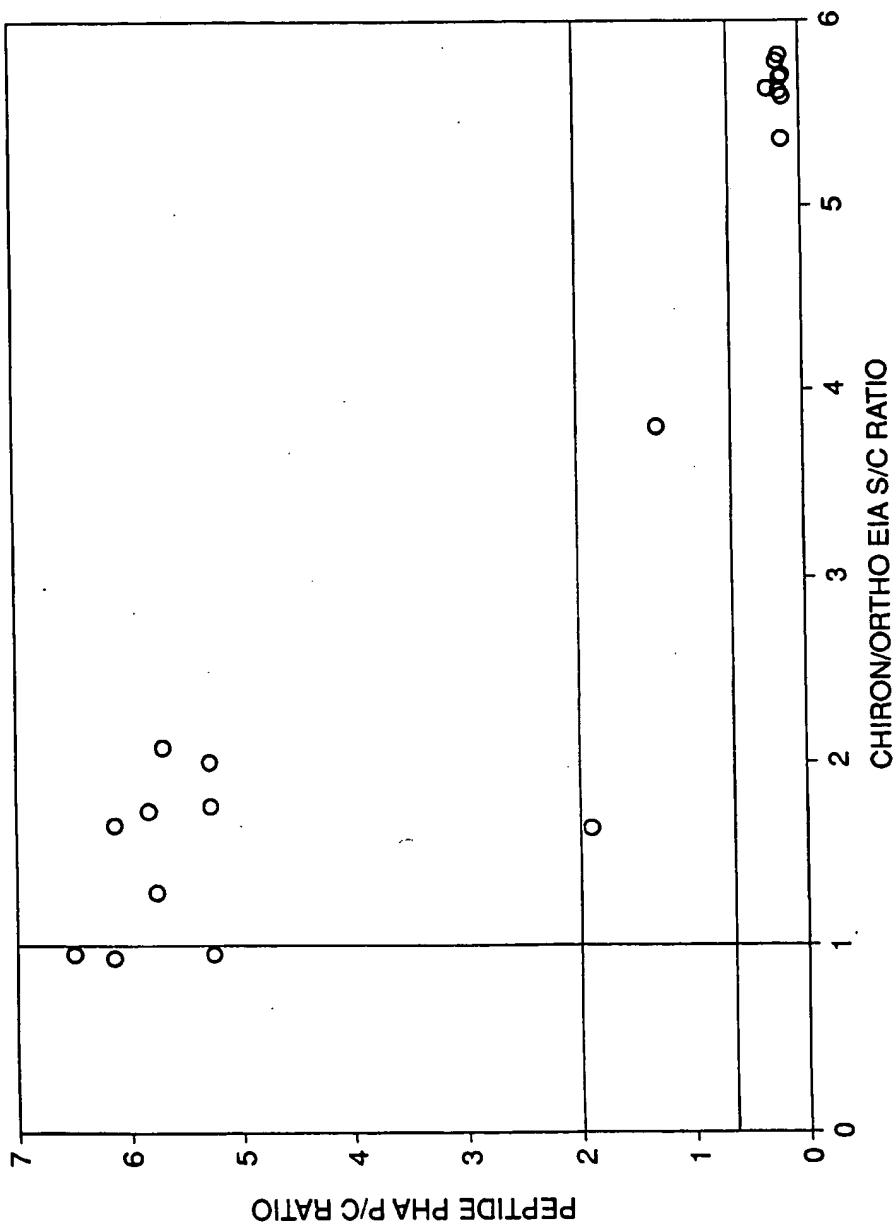


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FIG. 6.



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FIG. 7-1.

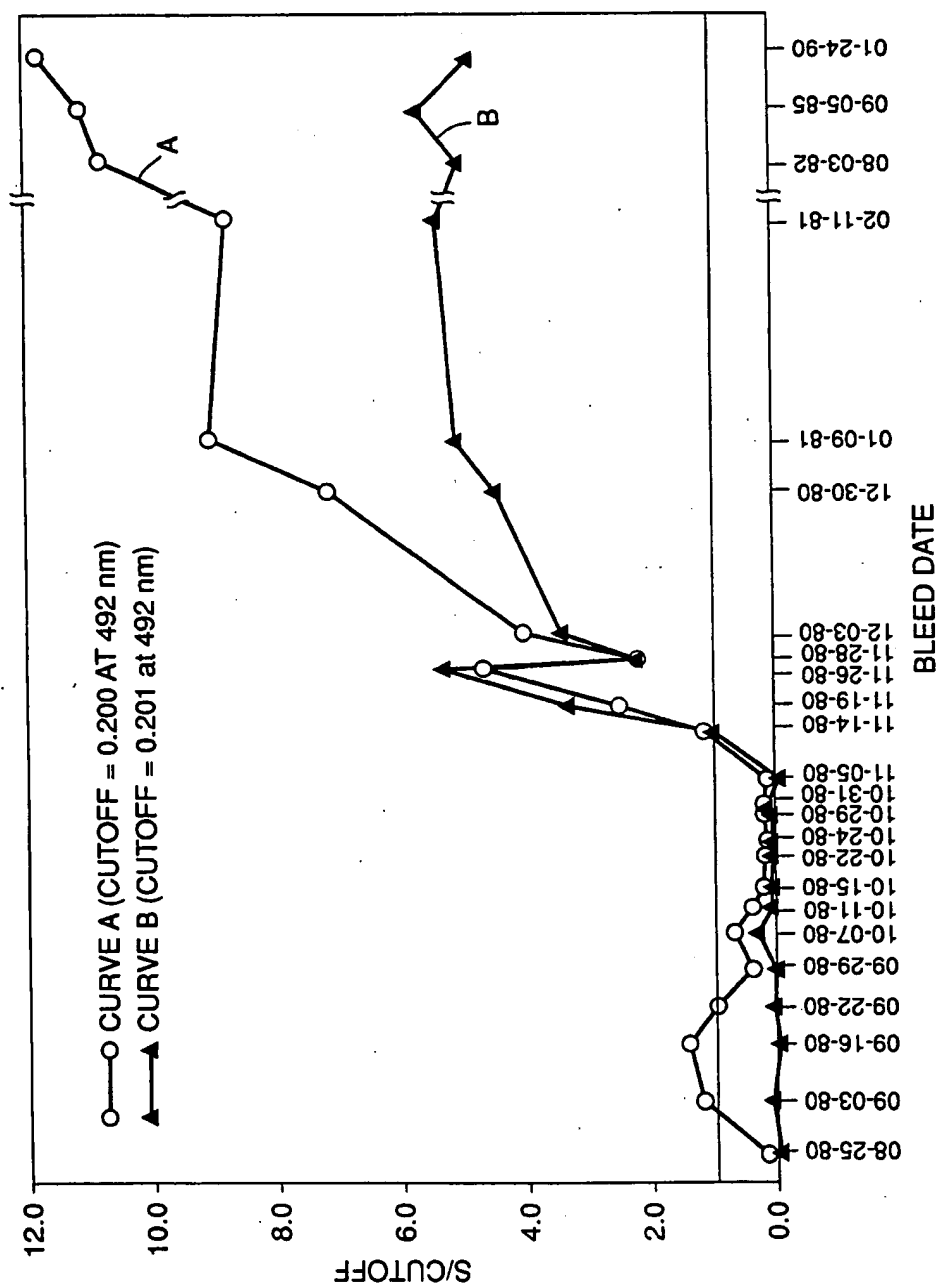
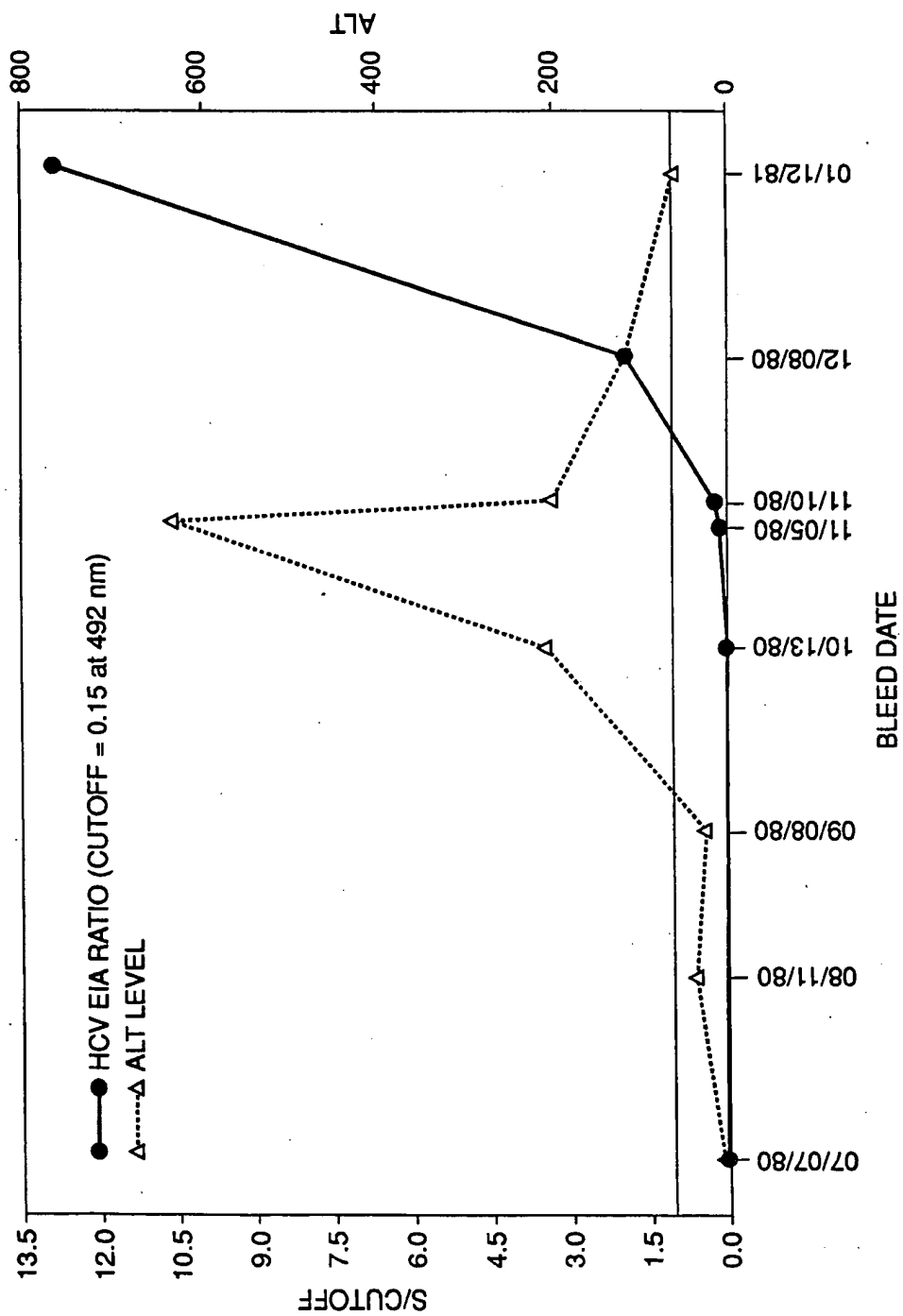


FIG. 7-2.



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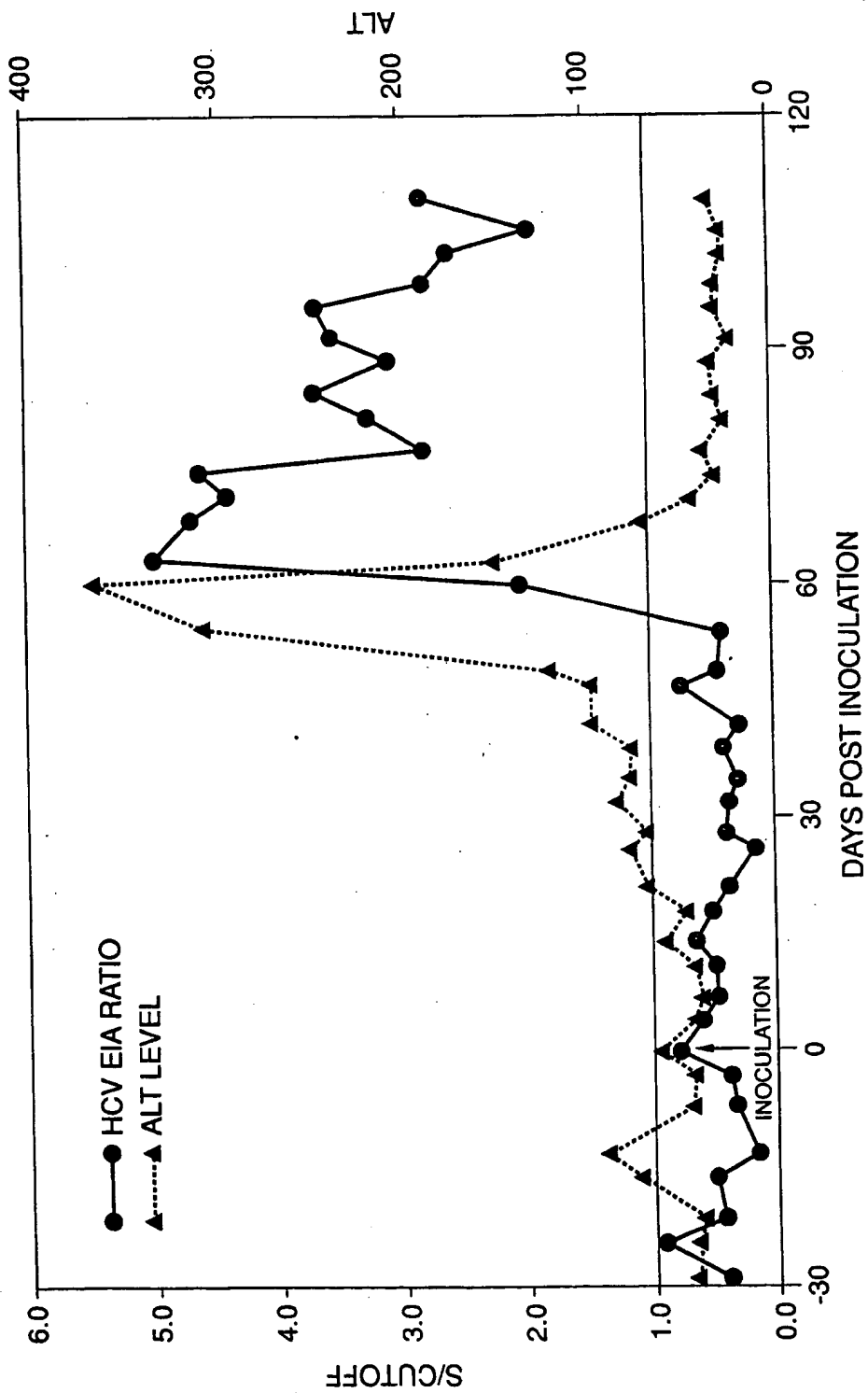
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None

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FIG. 7-3.



435 none  
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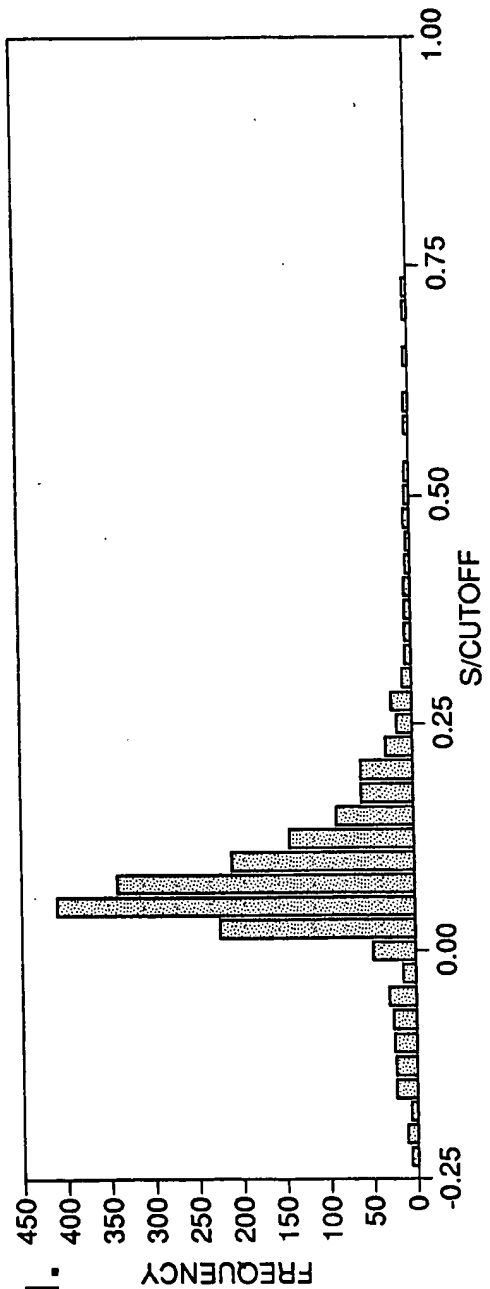


FIG. 8-1.

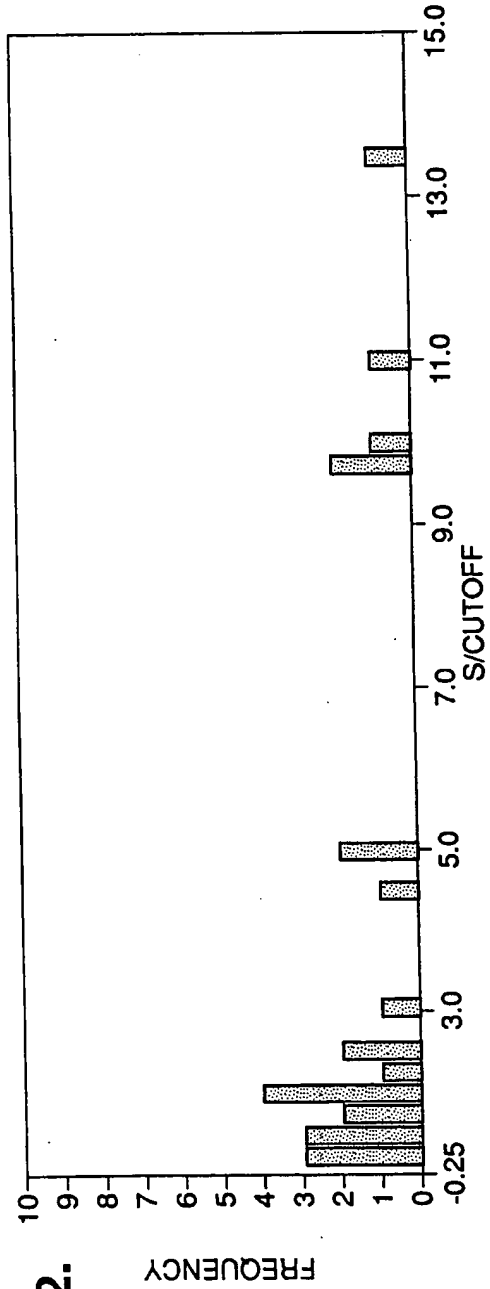


FIG. 8-2.

FIG. 9.

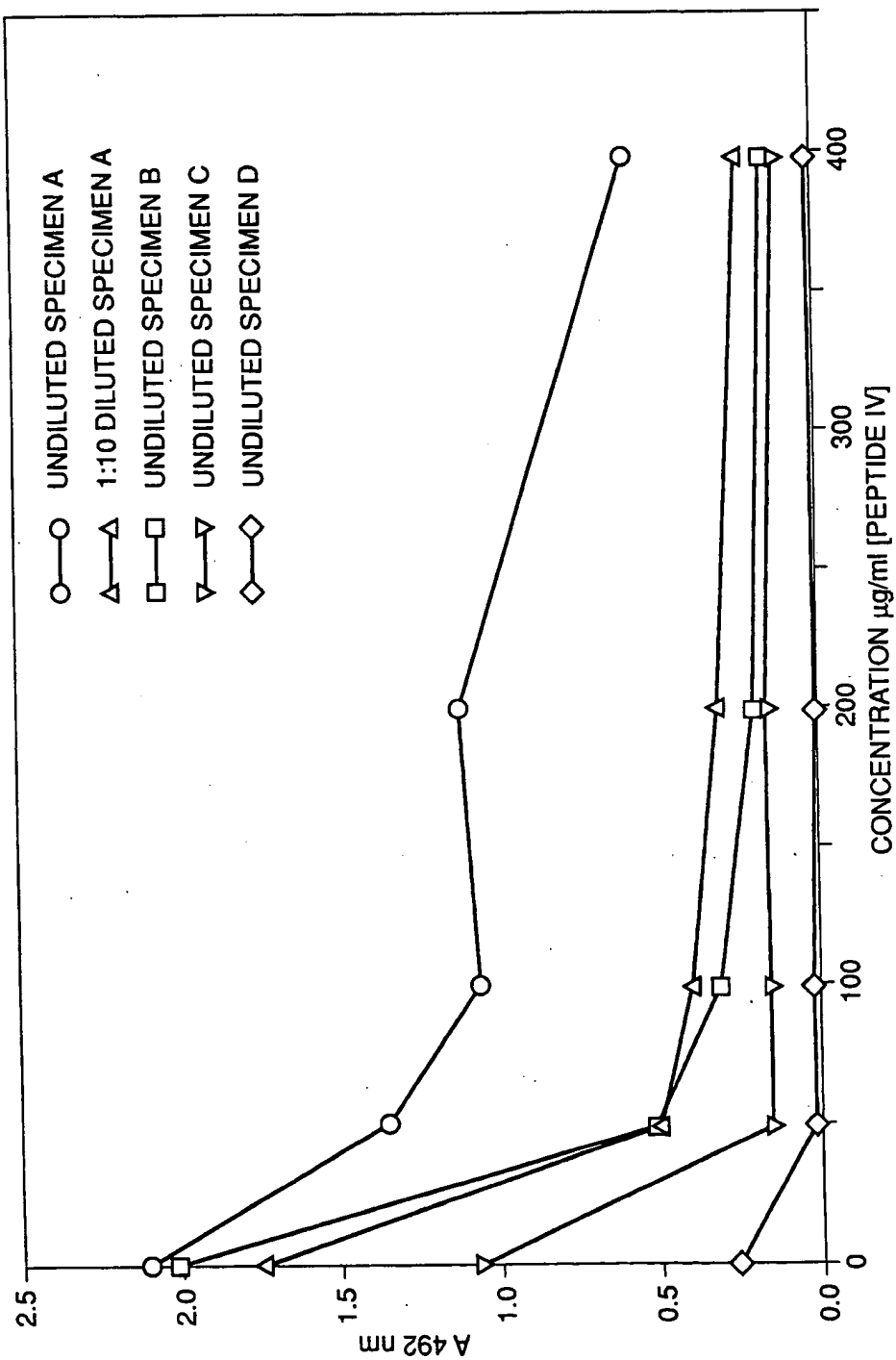
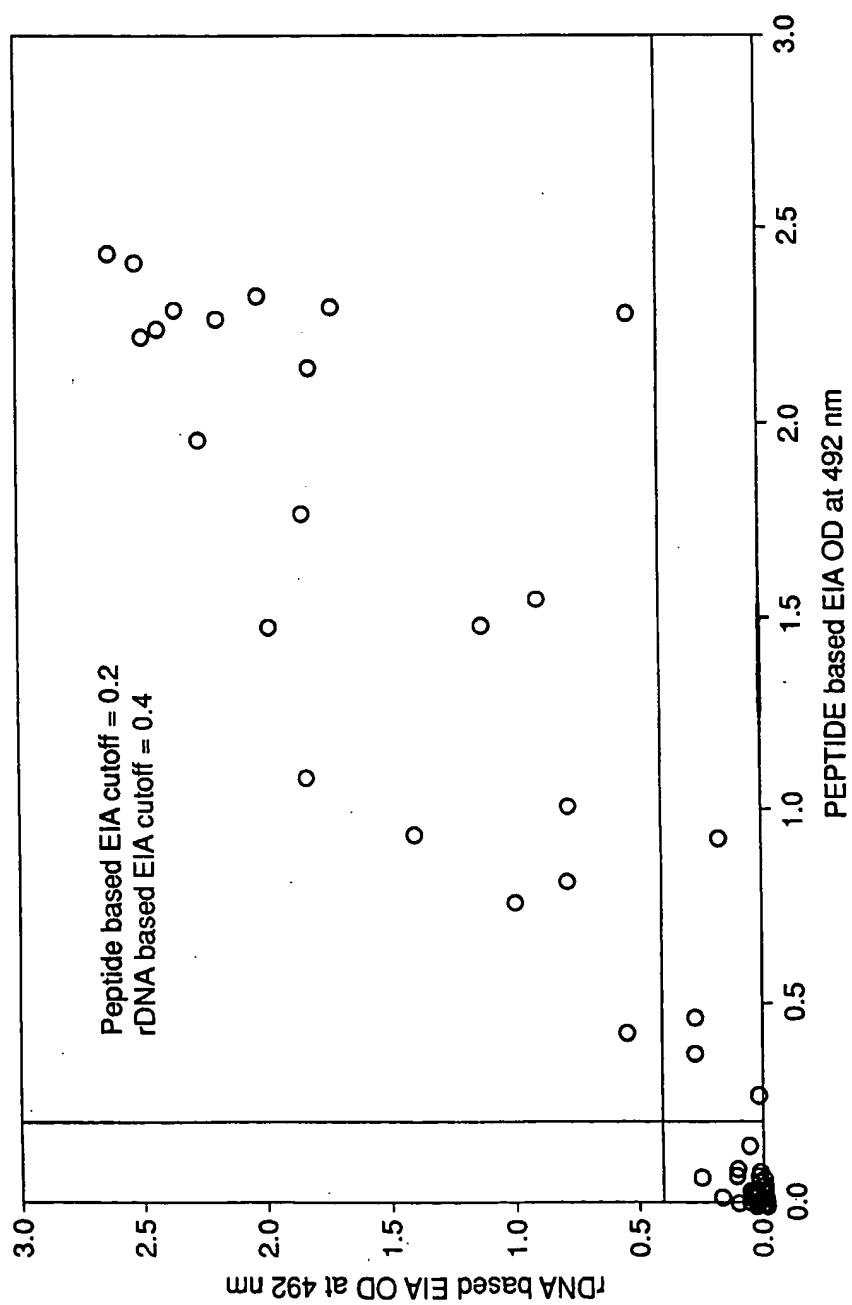


FIG. 10.



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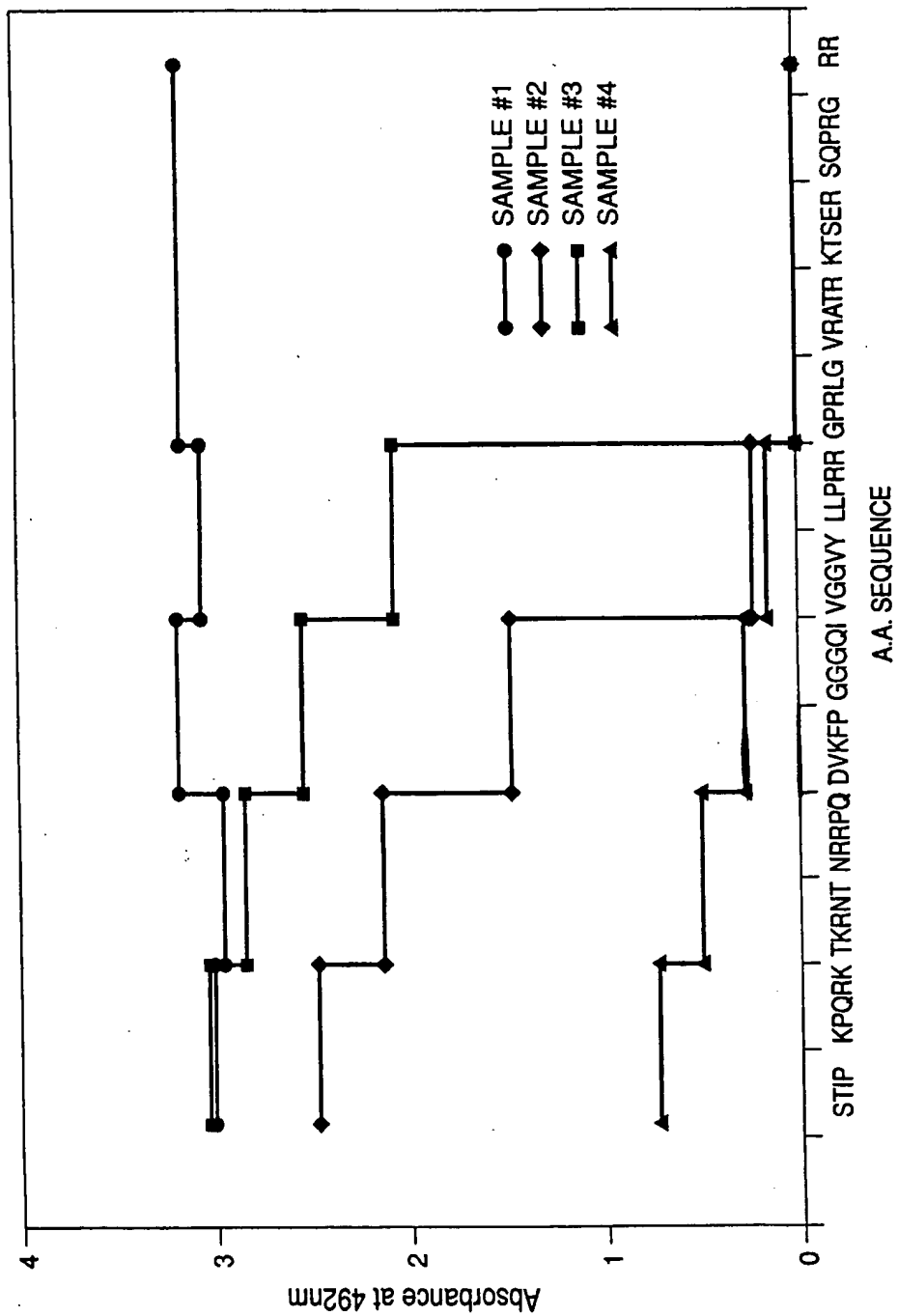
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FIG. 11-1.



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FIG. 11-2.

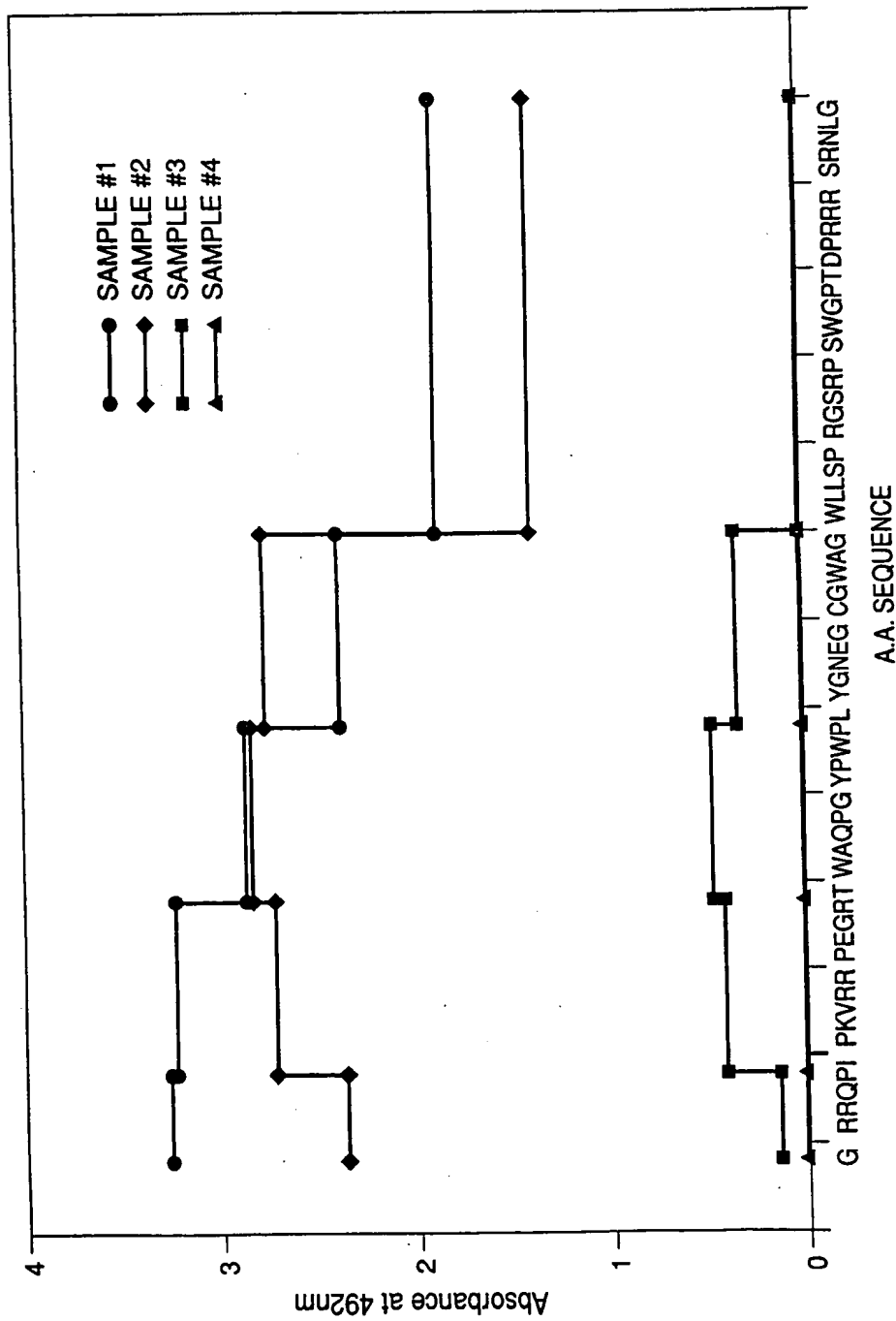
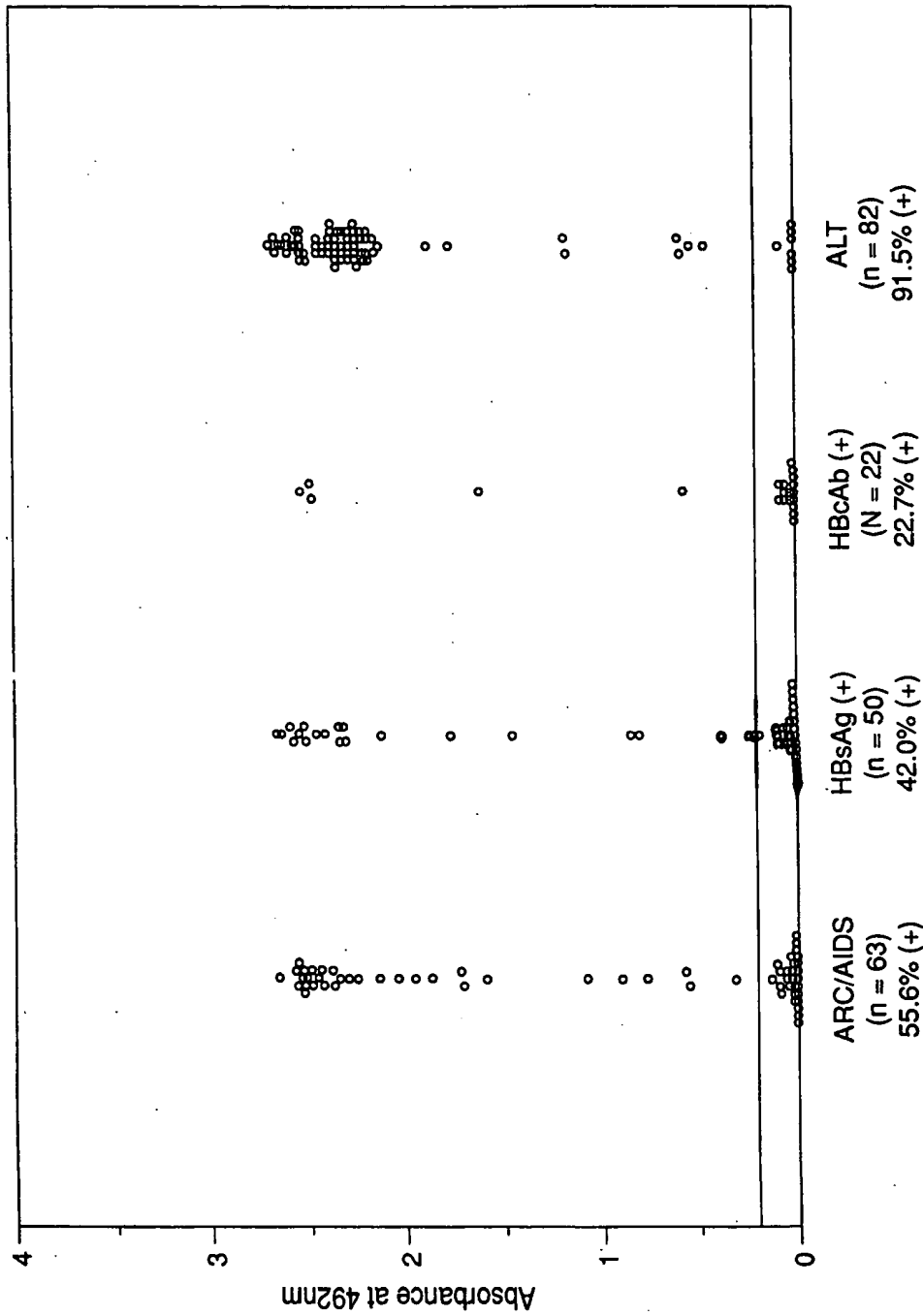


FIG. 12-1.



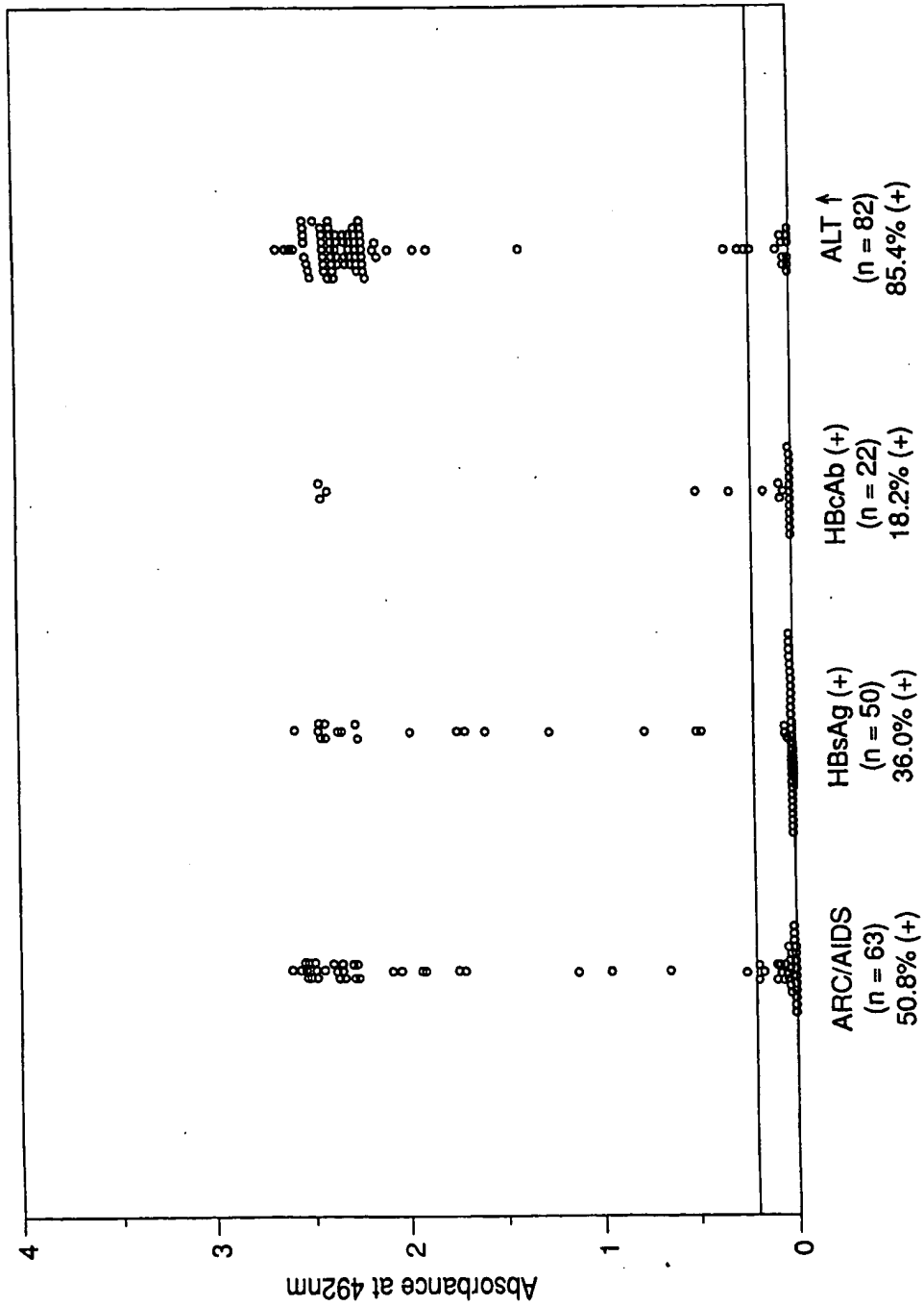
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FIG. 12-2.

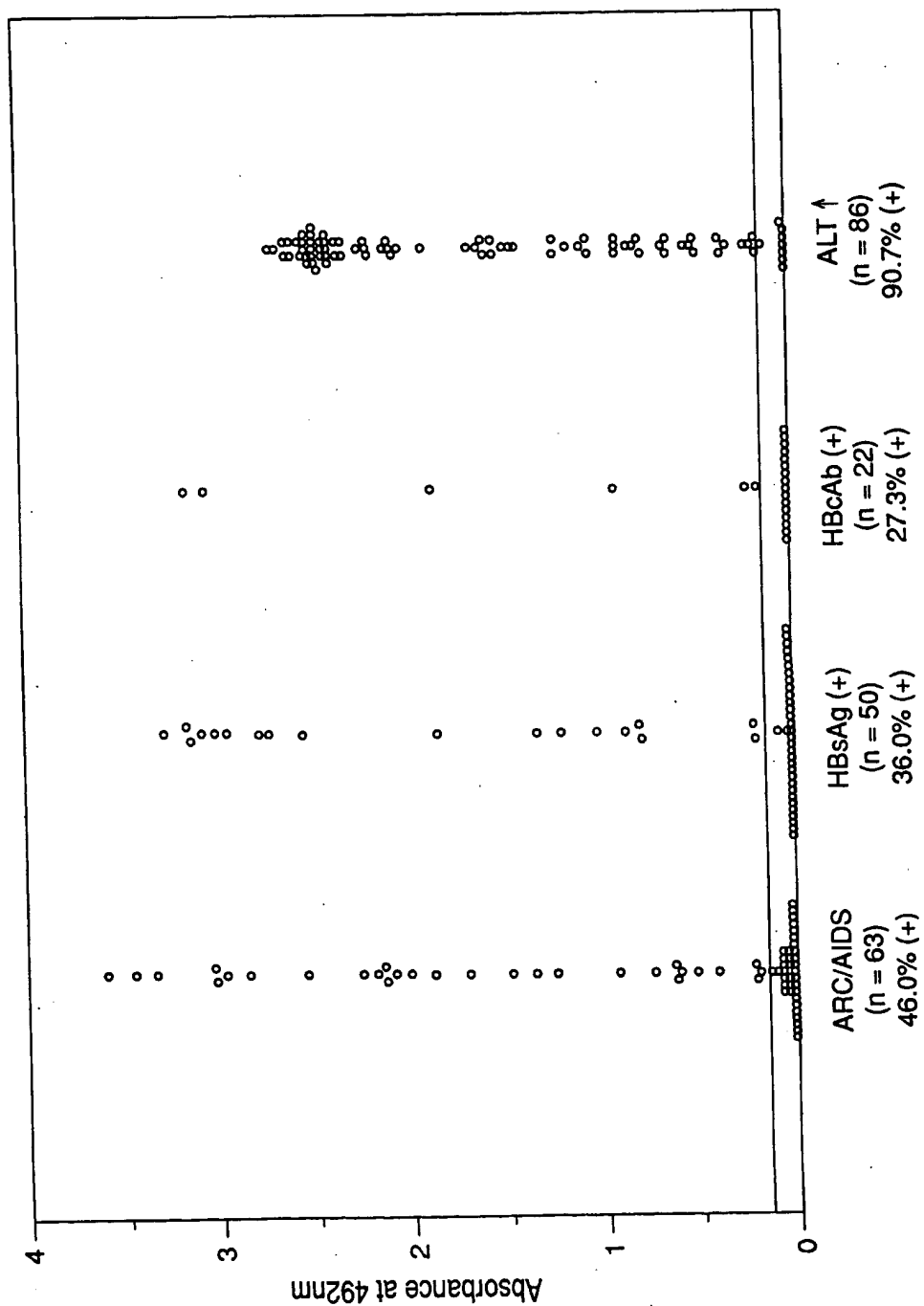


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FIG. 12-3.



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None

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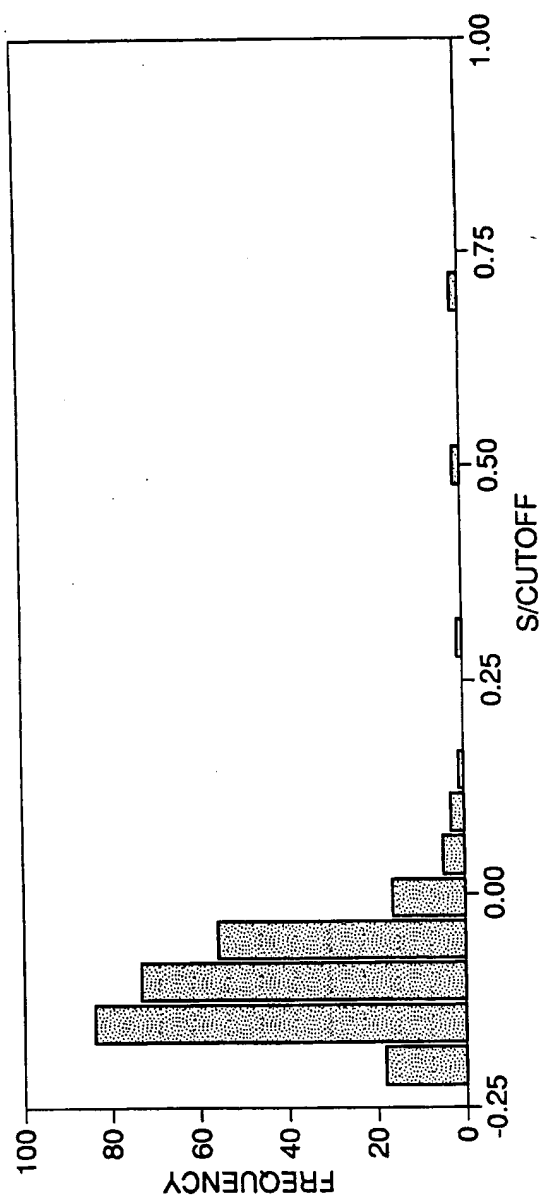


FIG. 13-1.

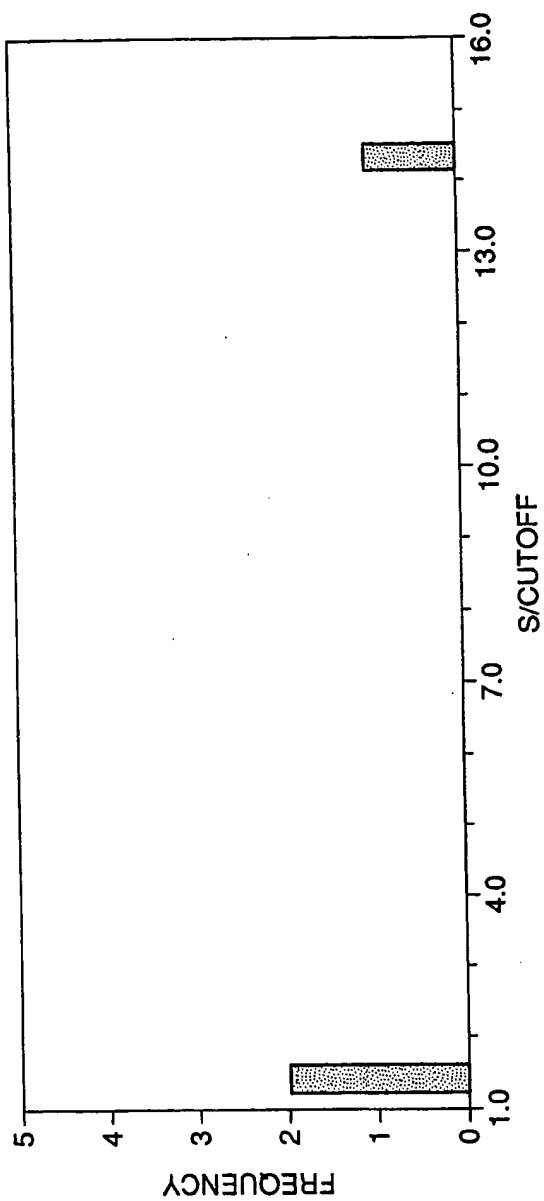


FIG. 13-2.

435 5 none

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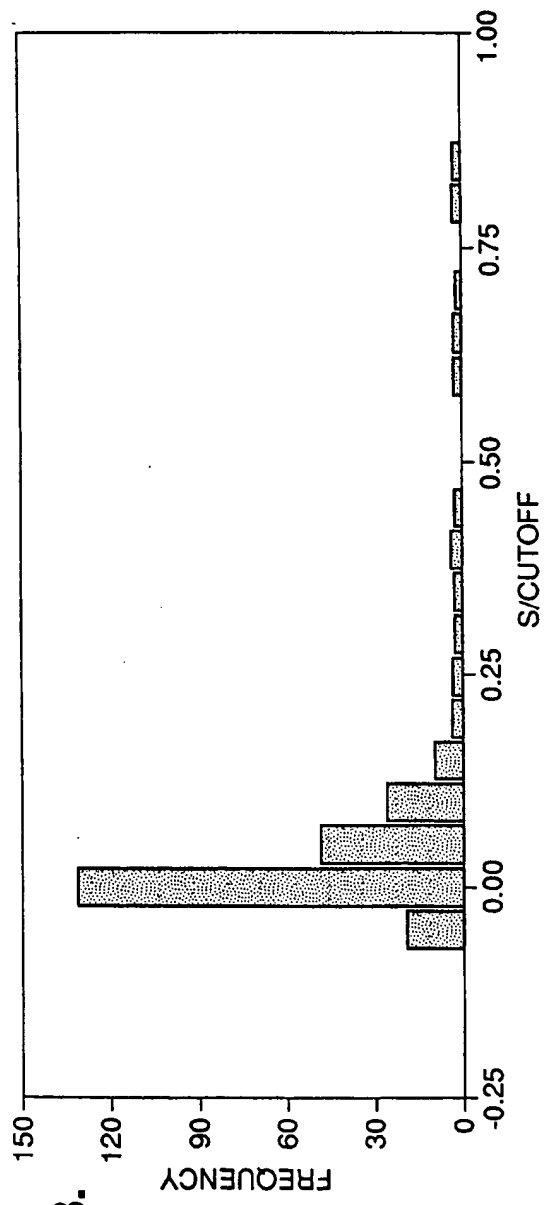


FIG. 13-3.

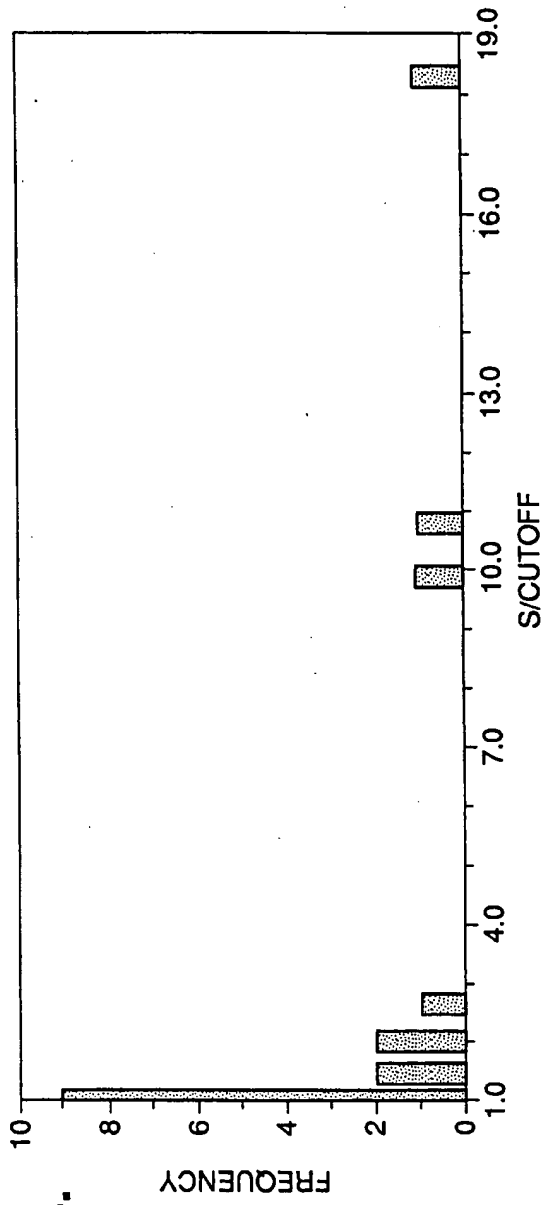


FIG. 13-4.

FIG. 13-5.

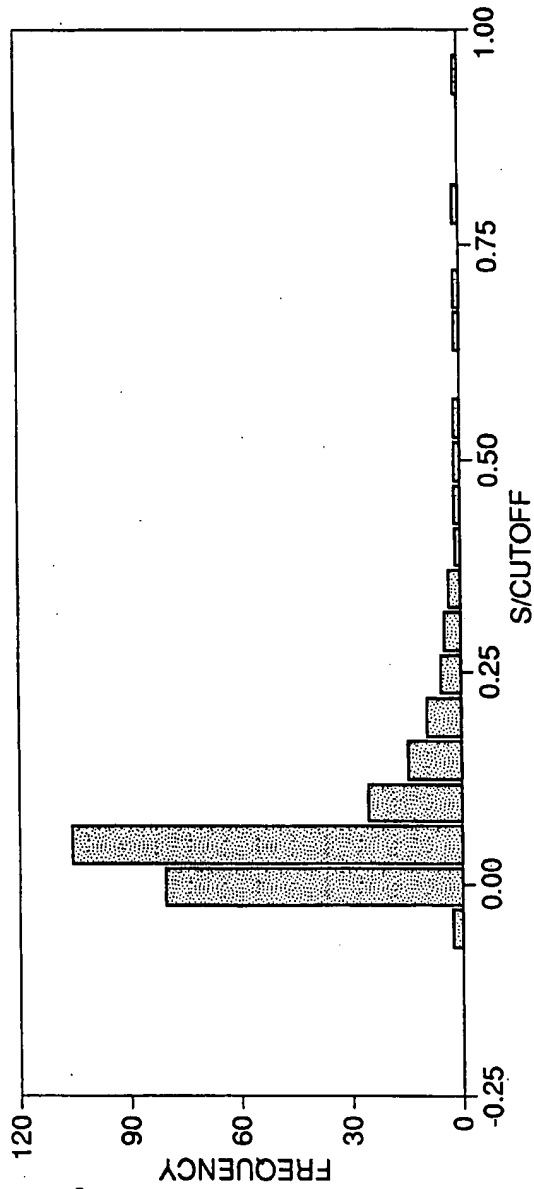
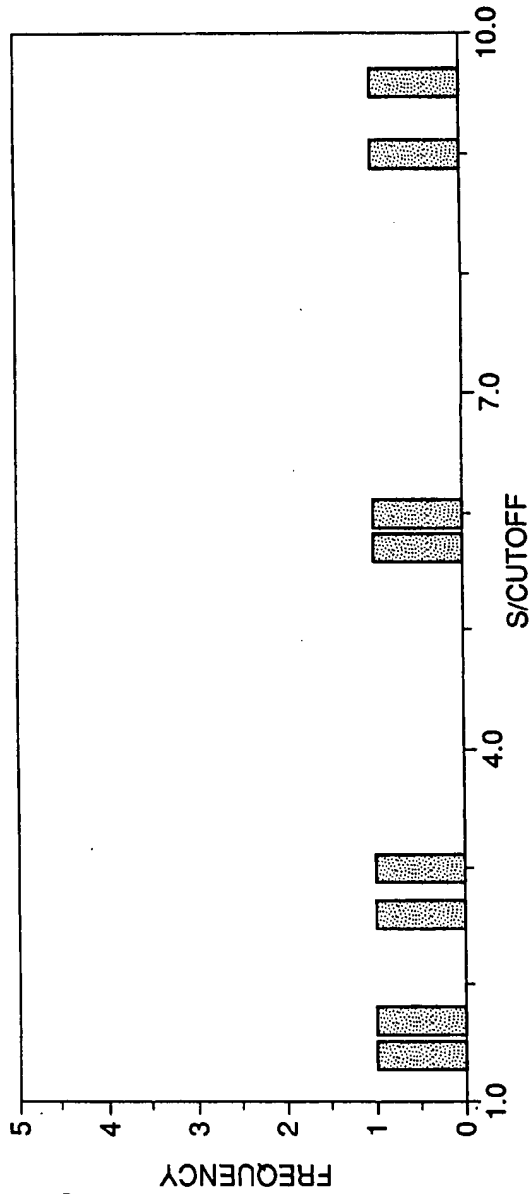


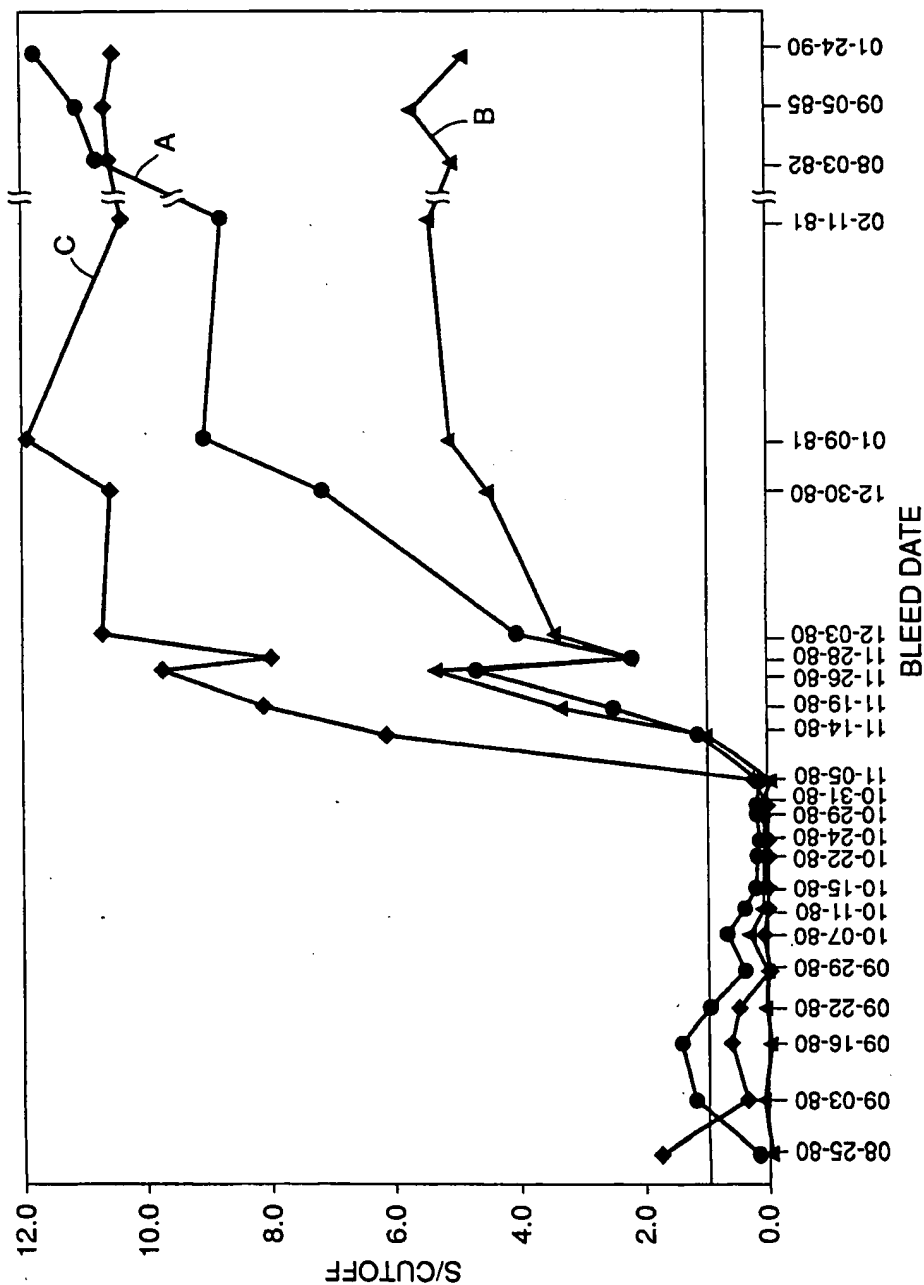
FIG. 13-6.



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FIG. 14-1.



● CURVE A (CUTOFF = 0.200 AT 492 nm)  
 ▲ CURVE B (CUTOFF = 0.201 AT 492 nm)  
 ◆ CURVE C (CUTOFF = 0.205 AT 492 nm)

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FIG. 14-2.

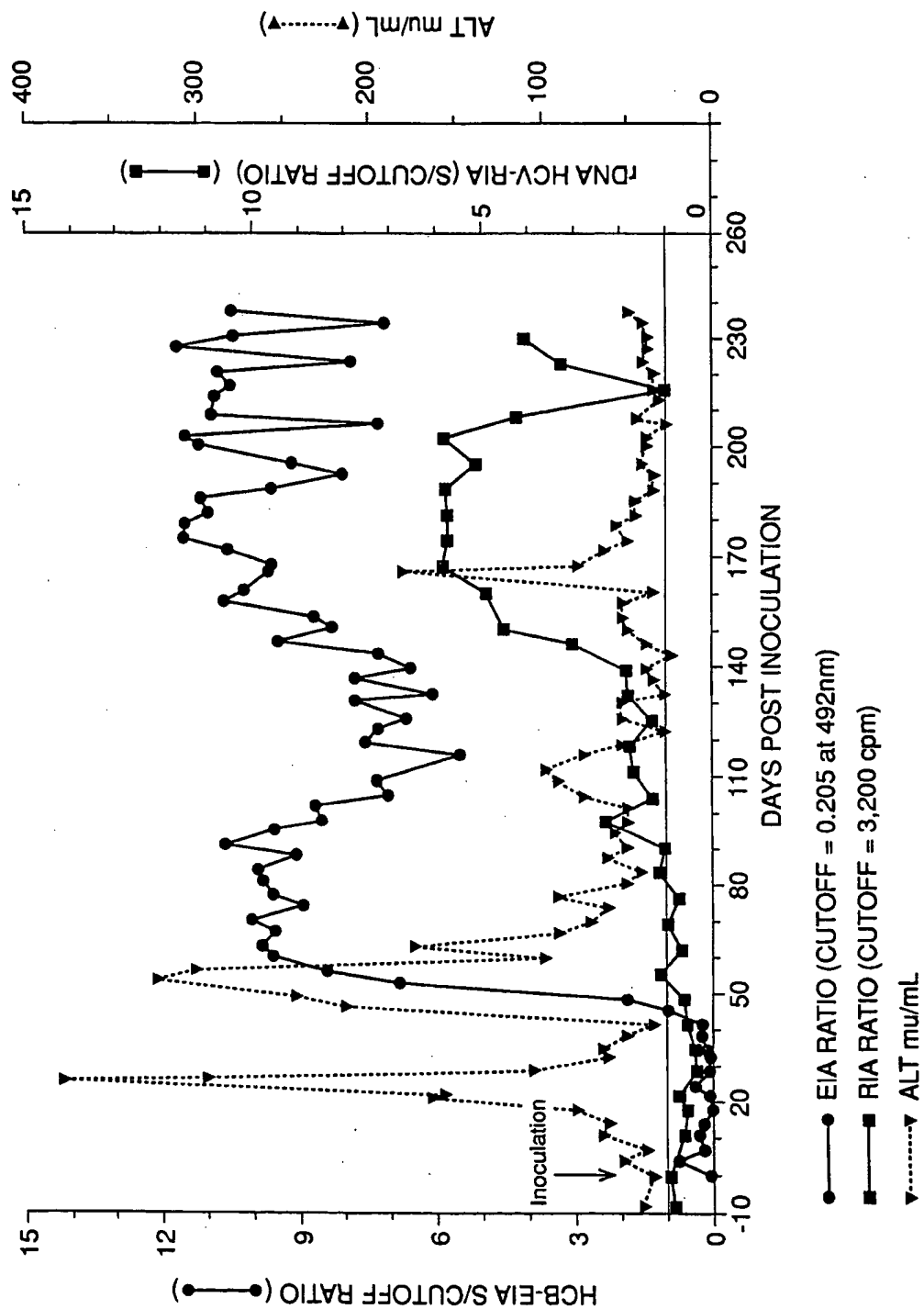


FIG. 15-1.

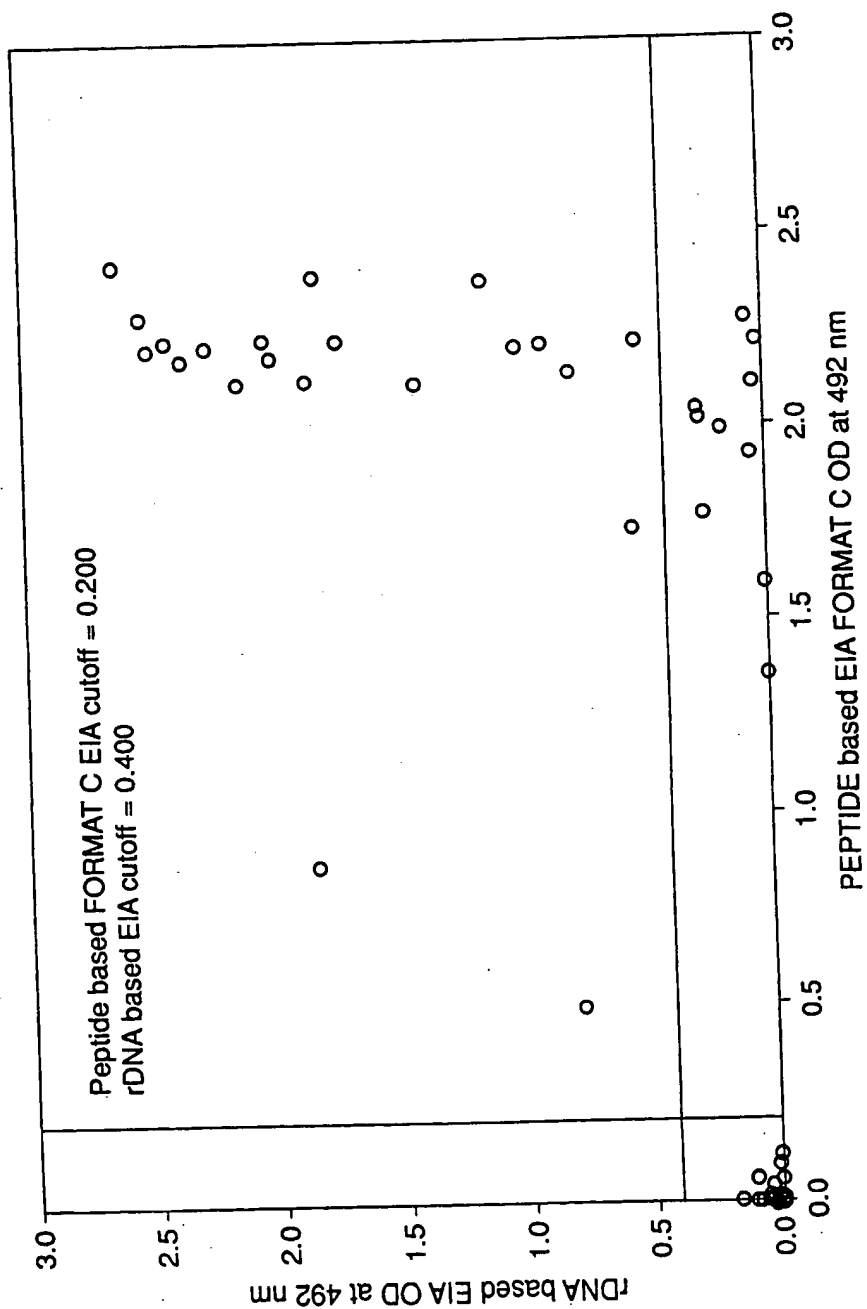
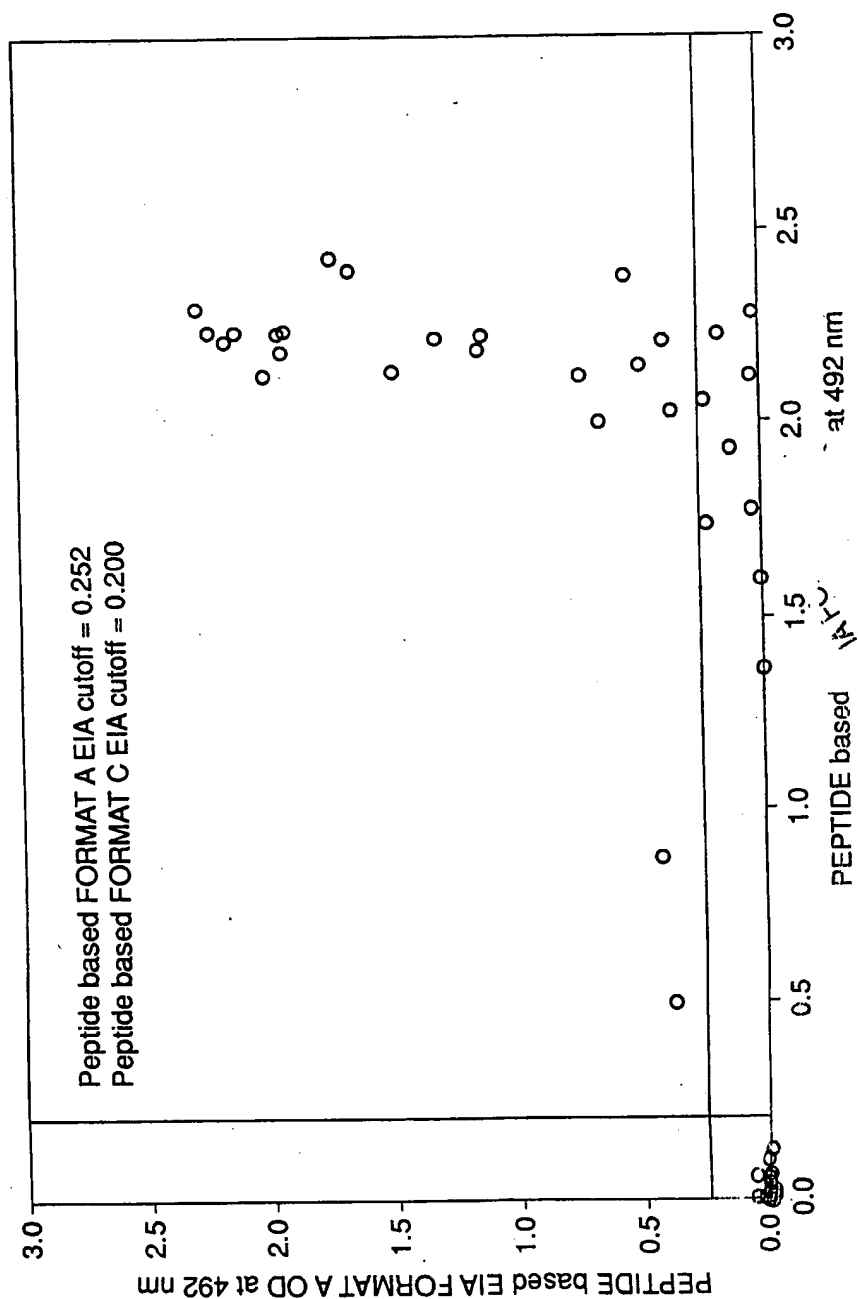


FIG. 15-2.



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Nov 2

Nov 2



PATENT

Docket No. 1151-4043

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Chang Yi Wang Group Art Unit: 189  
Serial No. : 07/558,799 Examiner: Lester Lee  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV  
DIAGNOSIS OF HCV INFECTION AND PREVENTION THEREOF AS VACCINES

AMENDMENT FEE TRANSMITTAL

HON. COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

Sir:

Transmitted herewith is an Amendment for the above-identified application.

☒ No additional fee is required.

☐ The additional fee has been calculated as shown below:

CLAIMS AS AMENDED

	Claims Remaining After Amendment	Highest No. Covered by Previous Payments	Present Extra	Rate	Additional Fee
Total Claims*	36	40	= -0-	x \$20.00	\$ -0-
Independent Claims	18	18	= -0-	x \$60.00	\$ -0-
Multiple Dependent Claim(s)	(If claims added by amendment include Multiple Dependent Claim(s) and there was no Multiple Dependent Claim(s) in application before amendment add \$200.00 to additional fee.)				\$ -0-
Total:					\$ -0-

☐ Verified Statement of "Small Entity" Status Under 37 CFR § 1.27  
filed \_\_\_\_\_ Reduced Fees Under 37 CFR § 1.9(f)  
(50% of total) paid herewith \$ \_\_\_\_\_

☐ Charge fee to Deposit Account No. 13-4500. Order No. \_\_\_\_\_  
A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

☒ The Commissioner is hereby authorized to charge any additional fees which may be required for this  
amendment, or credit any overpayment to Deposit Account No. 13-4500. Order No. 1151-4043.  
A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Includes all independent and single dependent claims and all claims referred to in multiple  
dependent claims. See 37 C.F.R. § 1.75(c).

Docket No. 1151-4043

- ☐        Page(s) of substitute Sequence Listing
- ☐        Computer disk(s) containing substitute Sequence Listing
- ☐ Statement under 37 C.F.R. § 1.825(b) that the computer and paper copies of the substitute Sequence Listing are the same.
- ☐ A check in the amount of \$            to cover the filing fee is attached.

Respectfully submitted,

MORGAN & FINNEGAN

Dated: September 26, 1991

By: Maria C. H. Lin  
Registration No. 29, 323

Mailing Address:

MORGAN & FINNEGAN  
345 Park Avenue  
New York, New York 10154  
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(212) 751-6849 Telecopier



PATENT  
U.S.S.N. 07/558,799

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Chang Yi Wang  
Serial No. : 07/558,799  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF  
ANTIBODIES TO HCV, DIAGNOSIS OF HCV INFECTION AND  
PREVENTIONS THEREOF AS VACCINES  
Group Art Unit : 189  
Examiner : Lester Lee

CERTIFICATE OF MAILING (37 C.F.R. 1.8a)

HON. COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

Sir:


I hereby certify that the attached AMENDMENT AND RESPONSE  
PURSUANT TO 35 C.F.R. 1.116 AFTER FINAL REJECTION (along with any paper(s)  
referred to as being attached or enclosed) and this Certificate of Mailing are being deposited  
with the United States Postal Service on the date shown below with sufficient postage as first  
class mail in an envelope addressed to the: BOX AF Commissioner of Patents and  
Trademarks, Washington, D.C. 20231.

Respectfully submitted,

MORGAN & FINNEGAN

Dated: September 26, 1991

By:

  
Maria C.H. Lin  
Registration No. 29,323

Mailing Address:

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345 Park Avenue  
New York, New York 10154  
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751-6849 Telecopier



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
07/558,799	07/26/90	WANG	C: 1151-4043

MORGAN & FINNEGAN  
345 PARK AVE.  
NEW YORK, NY 10154

EXAMINER	
LEE, L.	
ART UNIT	PAPER NUMBER
1809	9

DATE MAILED: 10/15/91

Below is a communication from the EXAMINER in charge of this application  
COMMISSIONER OF PATENTS AND TRADEMARKS

ADVISORY ACTION

☒ THE PERIOD FOR RESPONSE:

- ☐ is extended to run \_\_\_\_\_ from the date of the Final Rejection
- ☐ continues to run \_\_\_\_\_ from the date of the Final Rejection

☒ expires three months from the date of the final rejection or as of the mailing date of this Advisory Action, whichever is later. In no event however, will the statutory period for response expire later than six months from the date of the final rejection.

Any extension of time must be obtained by filing a petition under 37 CFR 1.136(a), the proposed response and the appropriate fee. The date on which the response, the petition, and the fee have been filed is the date of the response and also the date for the purposes of determining the period of extension and the corresponding amount of the fee. Any extension fee pursuant to 37 CFR 1.17 will be calculated from the date that the shortened statutory period for response expires as set forth above.

☐ Appellant's Brief is due in accordance with 37 CFR 1.192(a).

☒ Applicant's response to the final rejection, filed 9/30/91, has been considered with the following affect, but it is not deemed to place the application in condition for allowance:

1. ☒ The proposed amendments to the claim and/or specification will not be entered and the final rejection stands because:

- a. ☐ There is no convincing showing under 37 CFR 1.116(b) why the proposed amendment is necessary and was not earlier presented.
- b. ☒ They raise new issues that would require further consideration and/or search. (See Note).
- c. ☒ They raise the issue of new matter. (See Note).
- d. ☐ They are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal.
- e. ☐ They present additional claims without cancelling a corresponding number of finally rejected claims.

NOTE:

The new limitations as to immunoreactivity to antibodies to HIV relative to the peptide of at least 20% and the polymer definition appear to be new matter.

2. ☐ Newly proposed or amended claims \_\_\_\_\_ would be allowed if submitted in a separately filed amendment cancelling the non-allowable claims.

3. ☒ Upon the filing of an appeal, the proposed amendment ☐ will be ☒ will not be, entered and the status of the claims in this application would be as follows:

Allowed claims: 44-46 and 48-54

Claims objected to:

Claims rejected: 1, 3, 5-6, 8-11, 28, 30-33, 35-43, 47 and 55-64

However;

- a. ☐ The rejection of claims \_\_\_\_\_ on references is deemed to be overcome by applicant's response.
- b. ☐ The rejection of claims \_\_\_\_\_ on non-reference grounds only is deemed to be overcome by applicant's response.

4. ☐ The affidavit, exhibit or request for reconsideration has been considered but does not overcome the rejection.

5. ☐ The affidavit or exhibit will not be considered because applicant has not shown good and sufficient reasons why it was not earlier presented.

☐ The proposed drawing correction ☐ has ☐ has not been approved by the examiner.

☐ Other

Enter 2, 200  
LESLIE L. LEE  
PRIMARY PATENT EXAMINER  
ART UNIT 186-  
12913





1151-4043

PATENT  
U.S.S.N. 07/558,799

*Class*  
*11/6/91*  
*10/Amst*  
*D*  
*(10)*

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

CORRES. AND MAIL

**BOX AF**

Applicant(s) : Chang Yi Wang  
Serial No. : 07/558,799  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE  
DETECTION OF ANTIBODIES TO HCV,  
DIAGNOSIS OF HCV INFECTION AND  
PREVENTION THEREOF AS VACCINES  
Group Art Unit : 189  
Examiner : *✓* Lester Lee

*OK*  
*To order*  
*8/22/91*  
*11/18/91*  
*Plunkett*  
*11-22-91*  
*To Ex*  
*11-6-91*  
*8/22/91*

Hon. Commissioner of Patents and Trademarks  
Washington, D.C. 20231

AMENDMENT AND RESPONSE PURSUANT to 35 C.F.R. 1.116  
AFTER ADVISORY ACTION

Sir:

This is submitted in response to the Advisory Action dated October 15, 1991. The original response period was set to expire on November 15, 1991. Therefore, no extension fee is required at this time.

AMENDMENT

Please amend claims 1, 3, 5, 6, 8, 9, 30-33, and 35-42 as follows:

1 (Thrice Amended). A peptide composition comprising a peptide selected from the group consisting of Peptide I to IX each peptide with an amino acid sequence as follows:

*E1*  
*D*

- E1  
DL  
Cont
- (i) Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X  
(Peptide I)
- (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-X  
(Peptide II)
- (iii) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-  
Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X  
(Peptide III)
- (iv) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X  
(Peptide IV)
- (v) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-  
Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-  
Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X  
(Peptide V)
- (vi) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-  
Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-  
Thr-X  
(Peptide VI)
- (vii) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-  
Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-  
Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-  
Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-  
Val-Ser-Pro-X  
(Peptide VII)
- (viii) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-  
Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-  
Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-  
Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-  
Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-  
Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-  
Arg-X  
(Peptide VIII)

and

(ix) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X

(Peptide IX)

wherein X is -OH or -NH<sub>2</sub>; and

- (x)
- a. an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved;
  - b. a segment of each of the above peptides or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least [20%] 1.3%;
  - c. a mixture of the above peptides or analogues of the peptides;
  - d. a conjugate of each of the peptides with carrier proteins, the conjugate having specific immunoreactivity to antibodies to HCV relative to the peptide of at least [20%] 1.3%; and
  - e. a polymer of each of the peptides comprising a branching dimer, tetramer, or octomer of the peptide on a mono, tri, or hepta lysine core respectively.

3 (Thrice Amended). A peptide composition according to Claim 1 wherein the peptide comprises a segment of Peptide II and has an amino acid sequence selected from the group consisting of:

- E2  
D2
- (i) Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IIC)
  - (ii) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IID)
  - (iii) Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IIE)
  - (iv) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IIF)

wherein X is -OH or -NH<sub>2</sub> and an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved.

43 (Thrice Amended). A peptide composition according to Claim 1 wherein the peptide comprises a segment of Peptide III and has an amino acid sequence selected from the group consisting of:

- D3
- (i) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;  
(Peptide IIIC)

(ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;  
(Peptide IIID)

(iii) Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;  
(Peptide IIIE)

3  
D  
2  
con

wherein X is - OH or -NH<sub>2</sub> and an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved.

5 (Thrice Amended). A peptide composition according to Claim <sup>4</sup> wherein the peptide has an amino acid sequence as follows:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;  
(Peptide IIID)

wherein X is -OH or -NH<sub>2</sub> or [an] an analogue of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved.

4 (Thrice Amended). A peptide composition according to Claim 1 wherein the peptide comprises a segment of Peptide VIII and has an amino acid sequence selected from the group consisting of:

D4

- D4  
cont
- (i) Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIIID)
  - (ii) Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIIIC)
  - (iii) Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIIIB)
  - (iv) Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;  
(Peptide VIIIA)

wherein X is -OH or -NH<sub>2</sub> or an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved.

3 (Thrice Amended). A peptide composition according to Claim 1 wherein the peptide comprises a segment of Peptide IX and has an amino acid sequence selected from the group consisting of:

- (i) Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;  
(Peptide IXD)
- (ii) Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;  
(Peptide IXC)

(iii) Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

(Peptide IXB)

(iv) Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

(Peptide IXA)

wherein X is -OH or -NH<sub>2</sub> or an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved.

8  
10 (Twice Amended). A peptide composition according to Claim 1 wherein the peptide has an amino acid sequence as follows:

Ser-Thr-Pro-Lys-Pro-Gln-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X

wherein X is -OH or -NH<sub>2</sub> or an analogue[.] of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide, and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved; and a segment of the above peptide having specific immunoreactivity to antibodies to HCV relative to the peptide of at least [20%] 1.3%.

9  
11 (Twice Amended). A peptide composition according to Claim 1 wherein the peptide has an amino acid sequence as follows:

D4  
concluded

Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is -OH or -NH<sub>2</sub>, ~~an~~ <sup>E</sup>analogue[.] of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide, and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved; and a segment of the above peptide or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least [20%] 1.3%.

11  
30 (Twice Amended). An ELISA test kit according to claim 28 wherein the solid phase is coated with a peptide composition comprising a segment of Peptide II and having an amino acid sequence selected from the group consisting of:

- D5
- (i) Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
  - (ii) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
  - (iii) Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;
  - (iv) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-



Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

- (v) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-  
Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-  
Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-  
Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-  
Leu-Gly-Leu-X;

wherein X is -OH or -NH<sub>2</sub>, and an analogue of each of the  
above peptides having an amino acid sequence derived from a  
strain/isolate of HCV in a region corresponding to the  
peptide and having specific immunoreactivity to antibodies  
to HCV relative to the peptide [of at least 20%] that is  
substantially preserved.

31<sup>12</sup> (Twice Amended). An ELISA test kit according to  
claim 28 wherein solid phase is coated with a peptide having  
an amino acid sequence:

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

wherein X is OH or -NH<sub>2</sub> and an analogue of the above peptide  
having an amino acid sequence derived from a strain/isolate  
of HCV in a region corresponding to the peptide and having  
specific immunoreactivity to antibodies to HCV relative to  
the peptide [of at least 20%] that is substantially  
preserved.

32<sup>13</sup> (Twice Amended). An ELISA test kit according to  
claim 28 wherein the solid phase is coated with a peptide  
composition comprising a segment of Peptide III having an  
amino acid sequence selected from the group consisting of:

- D<sup>5</sup>  
Cont
- (i) Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;
  - (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;
  - (iii) Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

wherein X is -OH or -NH<sub>2</sub> and an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved.

114  
23 (Twice Amended). An ELISA test kit according to claim 32 wherein the peptide is:

- (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved.

D<sup>6</sup>

115 (Twice Amended). An ELISA test kit according to Claim 28 wherein solid phase is coated with a peptide composition comprising a segment of Peptide VIII having an amino acid sequence selected from the group consisting of:

- (i) Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-

Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;

- (ii) Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;
- (iii) Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;
- (iv) Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved.

6  
D  
cont

14  
38 (Twice Amended). An ELISA test kit according to Claim 28 wherein solid phase is coated with a peptide composition comprising a segment of Peptide IX having an amino acid sequence selected from the group consisting of:

- (i) Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (ii) Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (iii) Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;
- (iv) Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is -OH or -NH<sub>2</sub> or an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved.

27 (Twice Amended). An ELISA test kit according to Claim 26 wherein the solid phase is coated with a peptide composition comprising a peptide having an amino acid sequence as follows:

Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-  
Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-  
Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-  
Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-  
Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-  
Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-  
Arg-X

wherein X is -OH or -NH<sub>2</sub> or an analogue of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide, and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved; and a segment of the above peptide or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least [20%] 1.3%.

28 (Twice Amended). An ELISA test kit according to Claim 28 wherein the solid phase is coated with a peptide composition comprising a peptide having an amino acid sequence as follows:

Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-  
Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-

Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-  
Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-  
Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-  
Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-  
Gly-X;

wherein X is -OH or -NH<sub>2</sub> and an analogue of each of the  
above peptides having an amino acid sequence derived from a  
strain/isolate of HCV in a region corresponding to the  
peptide and having specific immunoreactivity to antibodies  
to HCV relative to the peptide [of at least 20%] that is  
substantially preserved.

19  
20 (Twice Amended). An ELISA test kit according to  
Claim 28 wherein the solid phase is coated with a peptide  
composition comprising a mixture of Peptides IIH and V,  
Peptides IIH and V having the following amino acid sequences  
respectively:

- (i) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-  
Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-  
Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-  
Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-  
Leu-Gly-Leu-X (IIH)
- (ii) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-  
Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-  
Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-  
Ala-Lys-His-Met-Trp-Asn-Phe-X; (V)

wherein X is -OH or -NH<sub>2</sub> or an analogue of each of the above  
peptides having an amino acid sequence derived from a  
strain/isolate of HCV in a region corresponding to the  
peptide and having specific immunoreactivity to antibodies  
to HCV relative to the peptide [of at least 20%] that is  
substantially preserved.

20  
20 (Twice Amended). An ELISA test kit according to  
Claim 28 wherein the solid phase is coated with a peptide  
composition comprising a mixture of Peptides IIF, IIID and

V, Peptide IIF, IIID and V having the following amino acid sequences respectively:

- (i) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X; (IIF)
- (ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X; (IIID)
- (iii) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X; (V)

wherein X is -OH or -NH<sub>2</sub> or an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved.

41 (Twice Amended). An ELISA test kit according to Claim 28 wherein the solid phase is coated with a peptide composition comprising a mixture of Peptides IIH, V and VIIIE, Peptide IIH, V and VIIIE having the following amino acid sequences respectively:

- (i) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X; (IIH)
- (ii) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X; (V)
- (iii) Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-X; (VIIIE)

wherein X is -OH or -NH<sub>2</sub> or [an] an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved.

42 (Twice Amended). An ELISA test kit according to Claim 28 wherein the solid phase is coated with a peptide composition comprising a mixture of Peptides VIIIE and IXD, Peptide VIIIE and IXD having the following amino acid sequences respectively:

- (i) Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X; (VIIIE)
- (ii) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X; (IXD)

wherein X is -OH or -NH<sub>2</sub> and an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide; and having specific immunoreactivity to antibodies to HCV relative to the peptide [of at least 20%] that is substantially preserved; a segment of each of the above peptide or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least [20%] 1.3%.

REMARKS

Claims 1, 3, 5, 6, 8, 9, 30-33, 35-42 and 47 to delete the limitation of "at least 20%" and insert the limitation "substantially preserved" for peptide analogues and "at least 1.3%" for peptide segments. Support for the amendment can be found in the specification for the amendment on Table 1, page 31; Table 7, page 32 and Table 2, page 40.

No new matter has been introduced. Entry of the amendment is requested.

The Examiner has issued an Advisory Action indicating that claims 44-46 and 48-54 are allowed.

The Examiner has rejected claims 1, 3, 5-6, 8-11, 28, 30-33, 35-43, 47 and 55-64. The reason for the rejection is stated as:

The new limitation as an immunoreactivity to antibodies to HCV relative to the peptide of at least 20% and the polymer definition appear to be new matter.

A telephone interview was had with the Examiner.

Applicant pointed out that the % relative immunoreactivity to antibodies to HCV of the segments are presented in Table 1 on page 31, Table 7 on page 32, and Table 2 on page 40. The range of relative immunoreactivities for various segments were described.

The relative immunoreactivities for: Peptide I segments are 3.0 to 45.6%; Peptide II segments are 3.1 to 101%; Peptide III segments 4.9 to 100%; Peptide VI segments



are 3.9 to 54.8%; Peptide V segments are 1.3 to 93.9%; Peptide VIII segments are 27.5 to 98.6% and Peptide IX segments are 23.8 to 49.5%.

Therefore, the range of relative immunoreactivities for the peptide segments ranged from 1.3 to 101%. Therefore, the limitation of at least 20% should not be considered new matter. However, the Examiner pointed out that at least 20% is not recited in haec verba, and suggest that the claims be amended to recite at least 1.3%. Per the Examiner's suggestions, Claims 1, 3, 5, 6, 8, 9, 30-33 and 35-42 have been so amended. The rejection is, therefore, moot.

The definition of analogues in the claims has been amended to recite that an analogue has an amino acid sequence corresponding to the peptide and has specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

Such substitutions, deletions and insertions for analogs were described on page 6-7 and page 27 of the specification. Moreover, pages 12-16 state that in such analogues the immunoreactivity recognizable by the antibodies to HCV is preserved. This assertion is proven by the Declaration of Dr. Chang Yi Wang submitted with the response of September 26, 1991. Therefore, no new matter is presented by the amendment.

The Examiner also objected to the definition of the polymer in Claim 1 as new matter.

Applicant wish to point out that in Example 7, the poly lysine core type polymers were described specifically. In fact, on page 58, at lines 18-24 these polymers and how

they are made are specifically disclosed. The specification states:

Thus, sequential propagation of Boc-Lys (Boc) will generate  $2^n$  reactive ends. The first level coupling of Boc-Lys (Boc) will produce two reactive amino ends as a bivalent carrier. The sequential generations of a second and third step with Boc-Lys (Boc) will produce carriers containing from (tetra-valent), and eight (octa-valent) reactive amino ends to which peptide antigens are attached.

These particular dimeric, tetrameric and octameric polymers on a mono, tri or hepta lysine core being described specifically, no new matter can said to be presented by the definition of polymers of the peptide in claim 1.

Based on this description, it cannot be said that the definition of polymer in claim 1 presents new matter. Therefore, the rejection of claim 1 on this ground should be withdrawn.

The Examiner indicated that the proposed amendment of September 26, 1991 will not be entered. Applicant hereby request that in view of the present amendment and the showing that no new matter has been presented, the amendments of September 26, 1991 amended hereby be entered.

Applicant also wish to point out that amendment of Claims 47, 55-64 where only to insert the correct or missing amino acid sequences and should be entered and allowed. The amendments were suggested by the Examiner and no new reasons were stated for rejection of these claims. It is believed that it was merely an oversight and that the claims 47 and 55-64 as amended are allowable.

The Examiner's courtesy and helpful suggestions during the telephone interview is deeply appreciated.

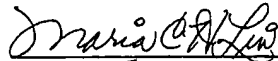
It is the Applicant's position that the claims as amended are allowable and an early allowance is requested.

Respectfully Submitted,

MORGAN & FINNEGAN

Dated: October 28, 1991

By:



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## PATENT

Docket No. 1151-4043

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Chang Yi Wang  
Serial No. : 07/558,799  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV, DIAGNOSIS OF HCV INFECTION AND PREVENTION THEREOF AS VACCINES

Group Art Unit: 189  
Examiner: Lester Lee

AMENDMENT FEE TRANSMITTAL

HON. COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

Sir:

Transmitted herewith is an Amendment for the above-identified application.

☒ No additional fee is required.☐ The additional fee has been calculated as shown below:CLAIMS AS AMENDED

	Claims Remaining After Amendment	Highest No. Covered by Previous Payments	Present Extra	Rate	Additional Fee
Total Claims*	36	40	= -0-	x \$20.00	\$ -0-
Independent Claims	18	18	= -0-	x \$60.00	\$ -0-
Multiple Dependent Claim(s)	(If claims added by amendment include Multiple Dependent Claim(s) and there was no Multiple Dependent Claim(s) in application before amendment add \$200.00 to additional fee.)				\$ -0-
Total:					\$ -0-

☐ Verified Statement of "Small Entity" Status Under 37 CFR § 1.27  
filed \_\_\_\_\_ Reduced Fees Under 37 CFR § 1.9(f)  
(50% of total) paid herewith

\$ \_\_\_\_\_

☐ Charge fee to Deposit Account No. 13-4500. Order No. \_\_\_\_\_  
A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

☒ The Commissioner is hereby authorized to charge any additional fees which may be required for this amendment, or credit any overpayment to Deposit Account No. 13-4500. Order No. 1151-4043.  
A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Includes all independent and single dependent claims and all claims referred to in multiple dependent claims. See 37 C.F.R. § 1.75(c).

Docket No. 1151-4043

- ☐ \_\_\_ Page(s) of substitute Sequence Listing
- ☐ \_\_\_ Computer disk(s) containing substitute Sequence Listing
- ☐ Statement under 37 C.F.R. § 1.825(b) that the computer and paper copies of the substitute Sequence Listing are the same.
- ☐ A check in the amount of \$ \_\_\_\_\_ to cover the filing fee is attached.

Respectfully submitted,

MORGAN & FINNEGAN

Dated: October 28, 1991

By:

Maria C. H. Lin

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1151-4043



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PATENT.

U.S.S.N. 07/558,799

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Chang Yi Wang  
Serial No. : 07/588,799  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF  
ANTIBODIES TO HCV, DIAGNOSIS OF HCV INFECTION AND  
PREVENTION THEREOF AS VACCINES  
Group Art Unit : 189  
Examiner : Lester Lee

CERTIFICATE OF MAILING (37 C.F.R. 1.8a)

HON. COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

Sir:

I hereby certify that the attached AMENDMENT & RESPONSE PURSUANT  
TO 35 CFR 1.116 AFTER ADVISORY ACTION (along with any paper(s) referred to as  
being attached or enclosed) and this Certificate of Mailing are being deposited with the  
United States Postal Service on the date shown below with sufficient postage as first-class  
mail in an envelope addressed to the: BOX AF, Commissioner of Patents and Trademarks,  
Washington, D.C. 20231.

Respectfully submitted,

MORGAN & FINNEGAN

Dated: October 28, 1991

By: 

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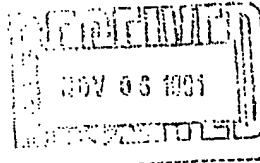
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PATENT  
U.S.S.N. 07/558,799

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Chang Yi Wang  
Serial No. : 07/558,799  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE  
DETECTION OF ANTIBODIES TO HCV,  
DIAGNOSIS OF HCV INFECTION AND  
PREVENTION THEREOF AS VACCINES  
Group Art Unit : 189  
Examiner : Lester Lee



Hon. Commissioner of Patents  
and Trademarks  
Washington, D.C. 20231

SUPPLEMENTAL AMENDMENT AND RESPONSE PURSUANT  
to 35 C.F.R. 1.116 AFTER ADVISORY ACTION

Sir:

This is to supplement the Amendment and Response  
submitted on October 28, 1991. The original response period  
was set to expire on November 15, 1991. Therefore, no  
extension fee is required at this time.

AMENDMENT

Please amend claims 1, 3, 10, 11, 37 and 42 and  
add new claim 62 as follows:

1 (Fourth Amendment). A peptide composition  
comprising a peptide selected from the group consisting of

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*White*  
*11-22-91*

*11/19/91*  
*11/19/91*  
*11/19/91*

*Sub 61*  
*E1*

Peptide I, III to IX, each peptide with an amino acid  
sequence as follows:

- (i) Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X  
(Peptide I)
- [(ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-X  
(Peptide II)]
- (ii[i]) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-  
Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X  
(Peptide III)
- [(iv)iii] Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X  
(Peptide IV)
- (iv) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-  
Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-  
Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X  
(Peptide V)
- (v[i]) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-  
Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-  
Thr-X  
(Peptide VI)
- (vi[i]) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-  
Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-  
Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-  
Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-  
Val-Ser-Pro-X  
(Peptide VII)
- (vii[i]) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-  
Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-  
Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-  
Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-  
Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-  
Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-  
Arg-X  
(Peptide VIII)

and



~~((ix)viii) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X~~

(Peptide IX)

wherein X is -OH or -NH<sub>2</sub>; and

- C1  
cont*
- (ix)
- a. an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved;
  - b. a segment of each of the above peptides or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least <sup>up to 101%</sup> [1.3%] 17.8%;
  - c. a mixture of the above peptides or analogues of the peptides;
  - d. a conjugate of each of the peptides with carrier proteins, the conjugate having specific immunoreactivity to antibodies to HCV relative to the peptide of at least [1.3%] 17.8%; and
  - e. a polymer of each of the peptides comprising a branching dimer, tetramer, or octomer of the peptide on a mono, tri, or hepta lysine core respectively.

92

3 (Thrice Amended). A peptide composition according to Claim [1]62 wherein the peptide comprises a segment of Peptide II and has an amino acid sequence selected from the group consisting of:

- (i) Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IIC)
- (ii) Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IID)
- (iii) Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IIE)
- (iv) Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
(Peptide IIF)

wherein X is -OH or -NH<sub>2</sub> and an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved.

5-13  
13

18 (Thrice Amended). A peptide composition according to Claim 1 wherein the peptide has an amino acid sequence as follows:

Ser-Thr-Pro-Lys-Pro-Gln-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X

3  
C  
cont

wherein X is -OH or -NH<sub>2</sub>, or an analogue of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide, and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved; and a segment of the above peptide having specific immunoreactivity to antibodies to HCV relative to the peptide of at least [1.3%] ~~17.8%~~.

9  
11 (Thrice Amended). A peptide composition according to Claim 1 wherein the peptide has an amino acid sequence as follows:

Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X;

wherein X is -OH or -NH<sub>2</sub>, an analogue of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide, and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved; and a segment of the above peptide or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least [1.3%] <sup>up to 10/9%</sup> ~~17.8%~~.

17  
28  
10  
C4

(Thrice Amended). An ELISA test kit according to Claim 28 wherein the solid phase is coated with a peptide composition comprising a peptide having an amino acid sequence as follows:

Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-  
Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-  
Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-  
Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-  
Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-  
Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-  
Arg-X

wherein X is -OH or -NH<sub>2</sub> or an analogue of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide, and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved; and a segment of the above peptide or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least [1.3%] <sup>up to 101%</sup> 17.6%.

<sup>22</sup>  
~~42~~ (Thrice Amended). An ELISA test kit according to Claim <sup>28</sup> wherein the solid phase is coated with a peptide composition comprising a mixture of Peptides VIIIE and IXD, Peptide VIIIE and IXD having the following amino acid sequences respectively:

- (i) Ser-Thr-Ile-pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X; (VIIIE)
- (ii) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X; (IXD)

wherein X is -OH or -NH<sub>2</sub> and an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide, and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially

ES  
conced  
F  
preserved; a segment of each of the above peptide or  
analogue thereof having specific immunoreactivity to  
antibodies to HCV relative to the peptide of at least  
[1.3%] <sup>up to 101%</sup> 17.8%.

Please add claim 62.

62. 2A peptide composition comprising Peptide II  
having an amino acid sequence as follows:

66  
(ii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-  
Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-  
Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-X  
(Peptide II)

and an analogue thereof having an amino acid sequence  
derived from a strain/isolate of HCV in a region  
corresponding to said peptide and having specific  
immunoreactivity to HCV relative to said peptide that is  
substantially preserved.

#### REMARKS

Claims 1, 3, 10, 11, 37 and 42 have been amended.  
The lower limitation of "at least 1.3%" is deleted and "at  
least 17.8%" for the peptide segments has been inserted.  
Support for the amendment can be found in the specification  
for the amendment on Table 1, page 31 and page 20 lines 21-  
22 of the specification and Figs. 1-1 to 1-4 in the  
drawings. Peptide II has been deleted from claim 1 and  
rewritten as claim 62.

No new matter has been introduced. Entry of the  
amendment is requested.

The Examiner has issued an Advisory Action  
indicating that claims 44-46 and 48-54 are allowed.

The Examiner has rejected claims 1, 3, 5-6, 8-11, 28, 30-33, 35-43, 47 and 55-61. The reason for the rejection is stated as:

The new limitation as an immunoreactivity to antibodies to HCV relative to the peptide of at least 20% and the polymer definition appear to be new matter.

A second telephone interview was had with the Examiner.

Applicant pointed out that the % relative immunoreactivity to antibodies to HCV of the segments are presented in Table 1 on page 31, Table 7 on page 32, and Table 2 on page 40. The range of relative immunoreactivities for various segments were described.

The relative immunoreactivities for: Peptide I segments are 3.0 to 45.6%; Peptide II segments are 3.1 to 101%; Peptide III segments 4.9 to 100%; Peptide VI segments are 3.9 to 54.8%; Peptide V segments are 1.3 to 93.9%; Peptide VIII segments are 27.5 to 98.6% and Peptide IX segments are 23.8 to 49.5%. However, on Table 1 and Figs. 1-1 to 1-4, certain epitopes have been underscored to show the degree of relative immunoreactivity that is contributed by the epitopes in the segments. According to the specification, page 20 lines 21-22, certain segments show marginal immunoreactivity when such epitopes are included in the peptide segment.

Based on this, the range of meaningful relative immunoreactivities for the peptide segments ranged from 17.8 to 101%. Therefore, the limitation of at least 17.8% should not be considered new matter. Applicant wishes to claim only those peptides which contain epitopes which contribute

meaningful immunoreactivity, which would be recognized by persons of skill in the art as being useful. It is believed that no new matter is presented by the Amendment. Therefore, rejection is now moot.

Peptide II has now been deleted from claim 1 and rewritten as claim 62. This is because only certain segments and analogues of Peptide II are desired to be claimed. See claim 3. No new matter is presented and claim 62 should be allowable.

The remaining rejections were responded to in the Amendment and Response filed October 28, 1991.

The Examiner's courtesy and helpful suggestions during the telephone interview is deeply appreciated.

It is the Applicant's position that the claims as amended are allowable and an early allowance is requested.

Respectfully Submitted,

MORGAN & FINNEGAN

Dated: November 5, 1991

By:



Maria C.H. Lin  
Registration No. 29,323

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9-H: 100  
PATENT

Docket No. 1151-4043

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Chang Yi Wang                      Group Art Unit: 189  
Serial No. : 07/558,799                      Examiner: Lester Lee  
Filed : July 26, 1990  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES  
TO HCV, DIAGNOSIS OF HCV INFECTION AND PREVENTION THEREOF  
AS VACCINES

AMENDMENT FEE TRANSMITTAL

HON. COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

Sir:

Transmitted herewith is an Amendment for the above-identified application.

☒ No additional fee is required.

☐ The additional fee has been calculated as shown below:

CLAIMS AS AMENDED

	Claims Remaining After Amendment	Highest No. Covered by Previous Payments	Present Extra	Rate	Additional Fee
Total Claims*	37	40	= 0	x \$20.00	\$ 0
Independent Claims	18	18	= 0	x \$60.00	\$ 0
Multiple Dependent Claim(s)	(If claims added by amendment include Multiple Dependent Claim(s) and there was no Multiple Dependent Claim(s) in application before amendment add \$200.00 to additional fee.)				\$ 0
Total:					\$ 0

☐ Verified Statement of "Small Entity" Status Under 37 CFR § 1.27  
filed \_\_\_\_\_ Reduced Fees Under 37 CFR § 1.9(f)  
(50% of total) paid herewith \$ \_\_\_\_\_

☐ Charge fee to Deposit Account No. 13-4500. Order No. \_\_\_\_\_  
A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

☒ The Commissioner is hereby authorized to charge any additional fees which may be required for this  
amendment, or credit any overpayment to Deposit Account No. 13-4500. Order No. 1151-4043  
A DUPLICATE COPY OF THIS SHEET IS ATTACHED.



Docket No. 1151-4043

- [ ]        Page(s) of substitute Sequence Listing
- [ ]        Computer disk(s) containing substitute Sequence Listing
- [ ] Statement under 37 C.F.R. § 1.825(b) that the computer and paper copies of the substitute Sequence Listing are the same.
- [ ] A check in the amount of \$            to cover the filing fee is attached.

Respectfully submitted,

MORGAN & FINNEGAN

Dated: November 5, 1991

By:

Maria C.H. Lin

Maria C.H. Lin

Registration No. 29,323

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UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
07/558,799	07/26/90	WANG	C 1151-4043

MORGAN & FINNEGAN  
345 PARK AVE.  
NEW YORK, NY 10154

LEE, L

EXAMINER	DATE OF ACTION
1811	12/F

1811

DATE MAILED: 11/26/91

### NOTICE OF ALLOWABILITY

#### PART I.

- ☒ This communication is responsive to the amendments filed 9/20/91, 10/31/91 and 11/16/91.
- ☒ All the claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice Of Allowance And Issue Fee Due or other appropriate communication will be sent in due course.
- ☒ The allowed claims are 1, 3, 5-6, 8-11, 28, 30-33, 35-55 and 59-62.
- ☒ The drawings filed on 9/30/91 are acceptable.
- ☐ Acknowledgment is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received. ☐ not been received. ☐ been filed in parent application Serial No. \_\_\_\_\_, filed on \_\_\_\_\_.
- ☒ Note the attached Examiner's Amendment.
- ☒ Note the attached Examiner Interview Summary Record, PTOL-413.
- ☐ Note the attached Examiner's Statement of Reasons for Allowance.
- ☐ Note the attached NOTICE OF REFERENCES CITED, PTO-892.
- ☐ Note the attached INFORMATION DISCLOSURE CITATION, PTO-1449.

#### PART II.

A SHORTENED STATUTORY PERIOD FOR RESPONSE to comply with the requirements noted below is set to EXPIRE THREE MONTHS FROM THE "DATE MAILED" indicated on this form. Failure to timely comply will result in the ABANDONMENT of this application. Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

- ☐ Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL APPLICATION, PTO-152, which discloses that the oath or declaration is deficient. A SUBSTITUTE OATH OR DECLARATION IS REQUIRED.
- ☐ APPLICANT MUST MAKE THE DRAWING CHANGES INDICATED BELOW IN THE MANNER SET FORTH ON THE REVERSE SIDE OF THIS PAPER.
  - ☐ Drawing informalities are indicated on the NOTICE RE PATENT DRAWINGS, PTO-948, attached hereto or to Paper No. \_\_\_\_\_. CORRECTION IS REQUIRED.
  - ☐ The proposed drawing correction filed on \_\_\_\_\_ has been approved by the examiner. CORRECTION IS REQUIRED.
  - ☐ Approved drawing corrections are described by the examiner in the attached EXAMINER'S AMENDMENT. CORRECTION IS REQUIRED.
  - ☐ Formal drawings are now REQUIRED.

Any response to this letter should include in the upper right hand corner, the following information from the NOTICE OF ALLOWANCE AND ISSUE FEE DUE: ISSUE BATCH NUMBER, DATE OF THE NOTICE OF ALLOWANCE, AND SERIAL NUMBER.

#### Attachments:

- Examiner's Amendment
- Examiner Interview Summary Record, PTOL-413
- Reasons for Allowance
- Notice of References Cited, PTO-892
- Information Disclosure Citation, PTO-1449

- Notice of Informal Application, PTO-152
- Notice re Patent Drawings, PTO-948
- Listing of Bonded Draftsmen
- Other

*Letter 2-see*  
LESTER L. LEE  
PRIMARY PATENT EXAMINER  
ART UNIT 1811  
1811

Examiner's Amendment

As per the telephone communication with applicant's attorney, Maria Lin, on November 18, 1991, the following extension of time and amendments to the claims were authorized:

This is a petition for an extension of time to December 15, 1991 please charge the fee for the extension of time to Deposit Account No. 13-4500. This extension of time is for order No. 1151-4043.

In the claims

Claim 1, line 71- the term "up to 101% " has been inserted after "17.8%".

Claim 11, line 18- the term "up to 101%" has been inserted after "17.8%".

Claim 37, line 18- the term "up to 101%" has been inserted after "17.8%".

Claim 42, line 26- the term "up to 101%" has been inserted after "17.8%".

Lee/vb  
November 21, 1991

*Lester L. Lee*  
LESTER L. LEE  
PRIMARY PATENT EXAMINER  
ART UNIT 188  
1811



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.

EXAMINER	
ART UNIT	PAPER NUMBER

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

All participants (applicant, applicant's representative, PTO personnel):

(1) Lester Lee (3) Chang Y. Wang

(2) Marie Ling (4) \_\_\_\_\_

Date of interview 9/5/91

Type: ☐ Telephonic ☒ Personal (copy is given to ☐ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☐ No. If yes, brief description: \_\_\_\_\_

Agreement ☐ was reached with respect to some or all of the claims in question. ☐ was not reached.

Claims discussed: All

Identification of prior art discussed: \_\_\_\_\_

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: \_\_\_\_\_

Agreed to that analogues, segments, mixtures and polymers (limit) limited to a property would be allowable.

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1-7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Lester L. Lee  
Examiner's Signature



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
077538, 779	07/26/90	WANG	1151-4043

MORGAN & FINNEGAN  
345 PARK AVE.  
NEW YORK, NY 10154

EXAMINER	
LEE L.	
ART UNIT	PAPER NUMBER
1313	

DATE MAILED:

EXAMINER INTERVIEW SUMMARY RECORD

11/26/91

All participants (applicant, applicant's representative, PTO personnel):

(1) Maria Lin (3) \_\_\_\_\_

(2) Lester L. Lee (4) \_\_\_\_\_

Date of interview 11/18/91

Type: ☒ Telephonic ☐ Personal (copy is given to ☐ applicant ☐ applicant's representative).

Exhibit shown or demonstration conducted: ☐ Yes ☐ No. If yes, brief description: \_\_\_\_\_

Agreement ☒ was reached with respect to some or all of the claims in question. ☐ was not reached.

Claims discussed: 1, 10, 37 and 42

Identification of prior art discussed: NONE

Description of the general nature of what was agreed to if an agreement was reached, or any other comments: \_\_\_\_\_

Agreed to insert upper limit of up to 101.  
Agreed to buy extension of time for 1 month.  
Case is allowable.

File # 13-4500 order # 1151-4043

(A fuller description, if necessary, and a copy of the amendments, if available, which the examiner agreed would render the claims allowable must be attached. Also, where no copy of the amendments which would render the claims allowable is available, a summary thereof must be attached.)

Unless the paragraphs below have been checked to indicate to the contrary, A FORMAL WRITTEN RESPONSE TO THE LAST OFFICE ACTION IS NOT WAIVED AND MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW (e.g., items 1-7 on the reverse side of this form). If a response to the last Office action has already been filed, then applicant is given one month from this interview date to provide a statement of the substance of the interview.

☐ It is not necessary for applicant to provide a separate record of the substance of the interview.

☐ Since the examiner's interview summary above (including any attachments) reflects a complete response to each of the objections, rejections and requirements that may be present in the last Office action, and since the claims are now allowable, this completed form is considered to fulfill the response requirements of the last Office action.

Lester L. Lee

Examiner's Signature



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

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Washington, D.C. 20231

MORGAN & FINNEGAN  
345 PARK AVE.  
NEW YORK, NY 10154

NOTICE OF ALLOWANCE  
AND ISSUE FEE DUE

- ☒ Note attached communication from the Examiner  
☐ This notice is issued in view of applicant's communication filed

SERIES CODE/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
07/558,799	07/26/90	037	LEE, L	1811 11/26/91
First Named Applicant	WANG, CHANG Y.			

TITLE OF INVENTION  
SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF ANTIBODIES TO HCV  
(AS AMENDED)

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
1 1151-0043	435-005,000	002	UTILITY	YES	\$525.00	02/26/92

**THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED.**

**THE ISSUE FEE MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED.**

**HOW TO RESPOND TO THIS NOTICE:**

**I. Review the SMALL ENTITY Status shown above.**

If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

- A. If the Status is changed, pay twice the amount of the FEE DUE shown above and notify the Patent and Trademark Office of the change in status, or  
B. If the Status is the same, pay the FEE DUE shown above.

If the SMALL ENTITY is shown as NO:

- A. Pay FEE DUE shown above, or  
B. File verified statement of Small Entity Status before, or with, payment of 1/2 the FEE DUE shown above.

II. Part B of this notice should be completed and returned to the Patent and Trademark Office (PTO) with your ISSUE FEE. Even if the ISSUE FEE has already been paid by a charge to deposit account, Part B should be completed and returned. If you are charging the ISSUE FEE to your deposit account, Part C of this notice should also be completed and returned.

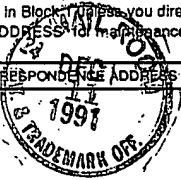
III. All communications regarding this application must give series code (or filing date), serial number and batch number. Please direct all communications prior to issuance to Box ISSUE FEE unless advised to the contrary.

**IMPORTANT REMINDER: Patents Issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees.**

PATENT AND TRADEMARK OFFICE COPY

## PART B - ISSUE FEE TRANSMITTAL

**MAILING INSTRUCTIONS:** This form should be used for transmitting the ISSUE FEE. Blocks 2 through 4 should be completed where appropriate. All further correspondence including the Issue Fee Receipt, the Patent, advanced orders and notification of maintenance fees will be mailed to addressee entered in Block 3 unless you direct otherwise, by: (a) specifying a new correspondence address in Block 3 below; or (b) providing the PTO with a separate "FEE ADDRESS" for maintenance fee notifications with the payment of Issue Fee or thereafter. See reverse for Certificate of Mailing.

1. CORRESPONDENCE ADDRESS	2. INVENTOR(S) ADDRESS CHANGE (Complete only if there is a change)
 MORGAN & FIRMENEGAN 445 PARK AVE. NEW YORK, NY 10154	INVENTOR'S NAME
	Street Address
	City, State and ZIP Code
	CO-INVENTOR'S NAME
	Street Address
	City, State and ZIP Code
	<input type="checkbox"/> Check if additional changes are on reverse side

PAPER TO BE ENTERED

SERIES CODE/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER AND GROUP ART UNIT	DATE MAILED
040 RP 12/18/91 07558799	07/28/90	037	LEE, J	1811 11/26/91
First Named Applicant: MORGAN, CHANNY Y.				

TITLE OF INVENTION  
KINETIC PARTIDES SPECIFIC FOR THE DETECTION OF ACTINOMYCETES TO HIV  
(SEE APPENDIX)

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SMALL ENTITY	FEE DUE	DATE DUE
1 1151 4042	015 005 000	002	UTILITY	1.0	1525.00	07/26/92

Express Mail No.: GB301069659US

3. Further correspondence to be mailed to the following:	4. For printing on the patent front page, list the names of not more than 3 registered patent attorneys or agents OR alternatively, the name of a firm having as a member a registered attorney or agent. If no name is listed, no name will be printed.
Maria C.H. Lin, Esq. Morgan & Firmnegan 345 Park Avenue New York, NY 10154	1 Maria C.H. Lin, Esq. 2 Morgan & Firmnegan 3

040 RP 12/18/91 07558799

DO NOT USE THIS SPACE

1 242 525.00 CK

5. ASSIGNMENT DATA TO BE PRINTED ON THE PATENT (print or type)	6a. The following fees are enclosed:
(1) NAME OF ASSIGNEE: United Biomedical, Inc.	<input checked="" type="checkbox"/> Issue Fee <input type="checkbox"/> Advanced Order - # of Copies (Minimum of 10)
(2) ADDRESS (City & State or Country) Lake Success, New York U.S.A.	6b. The following fees should be charged to:
(3) STATE OF INCORPORATION, IF ASSIGNEE IS A CORPORATION Delaware	DEPOSIT ACCOUNT NUMBER (Enclose Part C)
A. <input type="checkbox"/> This application is NOT assigned.	<input type="checkbox"/> Issue Fee <input type="checkbox"/> Advanced Order - # of Copies (Minimum of 10)
<input type="checkbox"/> Assignment previously submitted to the Patent and Trademark Office.	<input type="checkbox"/> Any Deficiencies in Enclosed Fees (Minimum of 10)
<input type="checkbox"/> Assignment is being submitted under separate cover. Assignments should be directed to Box ASSIGNMENTS.	The COMMISSIONER OF PATENTS AND TRADEMARKS is requested to apply the Issue Fee to the application identified above.
PLEASE NOTE: Unless an assignee is identified in Block 5, no assignee data will appear on the patent. Inclusion of assignee data is only appropriate when an assignment has been previously submitted to the PTO or is being submitted under separate cover. Completion of this form is NOT a substitute for filing an assignment.	(Signature of party in interest of record) (Date) Maria C.H. Lin Dec 11, 1991 Reg. No. 28,323
	NOTE: The Issue Fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office.

TRANSMIT THIS FORM WITH FEE-CERTIFICATE OF MAILING ON REVERSE

PART C - CHARGE TO DEPOSIT ACCOUNT



1. CORRESPONDENCE ADDRESS

MORRIS A. FURTHMAN  
100 PARK AVE.  
NEW YORK, NY 10034

SERIES CODE/SERIAL NO.	FILING DATE	TOTAL CLAIMS	EXAMINER/PROSECUTOR UNIT	DATE MAILED
11-11-99	11-11-99	1	11-11-99	11-11-99

First Named Applicant

MORRIS

CHARLES

TITLE OF INVENTION

SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION OF TUBERCULOSIS TO HIV  
(SEE ABSTRACT)

ATTY'S DOCKET NO.	CLASS-SUBCLASS	BATCH NO.	APPLN. TYPE	SP. FEE	FEE DUE	DATE DUE
11-11-99	11-11-99	11-11-99	11-11-99	11-11-99	11-11-99	11-11-99

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The COMMISSIONER OF PATENTS AND TRADEMARKS is requested to apply the Issue Fee to the application identified above.

(Signature of party in interest of record)

*Maria C. [Signature]*

(Date)

Dec. 11, 1999

NOTE: The Issue Fee will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the Patent and Trademark Office.

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2-222  
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D.C.  
1-21-91  
13/8



51-4043

PATENT  
U.S.S.N. 07/558,799

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Wang, Chang Yi  
Serial No. : 07/558,799  
Filed : July 26, 1991  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION  
OF ANTIBODIES TO HCV, DIAGNOSIS OF HCV  
INFECTION AND PREVENTION THEREOF AS VACCINES  
Group : 189  
Examiner : Lester Lee

Honorable Commissioner of  
Patents & Trademarks  
Washington, DC 20231

AMENDMENT PURSUANT TO 37 C.F.R. § 1.312

Sir:

Prior to the payment of the issue fee, applicant  
submits this amendment.

AMENDMENT

Please amend Claims 1, 10, 59, 60 and 61 as follows:

1 (Fifth Amendment). A peptide composition comprising a  
peptide selected from the group consisting of Peptide I, III to  
IX, each peptide with an amino acid sequence as follows:

- (i) Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-  
Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-  
Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-X  
(Peptide I)

-Express Mail No.:GB301069659US -

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- (ii) Cys-Val-Val-Ile-Val-Gly-Arg-Val-Val-Leu-Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-X  
(Peptide III)
- (iii) Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-X  
(Peptide IV)
- (iv) Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X  
(Peptide V)
- (v) Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-X  
(Peptide VI)
- (vi) Pro-Gly-Ala-Leu-Val-Val-Gly-Val-Val-Cys-Ala-Ala-Ile-Leu-Arg-Arg-His-Val-Gly-Pro-Gly-Glu-Gly-Ala-Val-Gln-Trp-Met-Asn-Arg-Leu-Ile-Ala-Phe-Ala-Ser-Arg-Gly-Asn-His-Val-Ser-Pro-X  
(Peptide VII)
- (vii) Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X  
(Peptide VIII)
- and
- (viii) Gly-Arg-Arg-Gln-Pro-Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-Arg-Arg-Ser-Arg-Asn-Leu-Gly-X  
(Peptide IX)

wherein X is -OH or -NH<sub>2</sub>; and

- (ix) a. an analogue of each of the above peptides having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved;

- b. a segment of each of the above peptides or analogue thereof having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 17.8% up to 101%;
- c. a mixture of the above peptides or analogues of the peptides;
- d. a conjugate of each of the peptides with carrier proteins, the conjugate having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 17.8% up to 101%; and
- e. a polymer of each of the peptides comprising a branching dimer, tetramer, or octomer of the peptide on a mono, tri, or hepta lysine core respectively.

<sup>8</sup> (Fourth Amendment). A peptide composition according to Claim 1 wherein the peptide has an amino acid sequence as follows:

Ser-Thr-Pro-Lys-Pro-Gln-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X

wherein X is -OH or -NH<sub>2</sub>, or an analogue of the above peptide having an amino acid sequence derived from a strain/isolate of HCV in a region corresponding to the peptide, and having specific immunoreactivity to antibodies to HCV relative to the peptide that is substantially preserved; and a segment of the above peptide having specific immunoreactivity to antibodies to HCV relative to the peptide of at least 17.8% up to 101%.

<sup>35</sup> (Twice Amended). An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

(i) [wherein the] a solid phase coated with a  
peptide composition [comprises] comprising a  
mixture of

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-  
Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-  
Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-  
Gln-His-Leu-Pro-Tyr-Ile-Glu-  
Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-  
Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;  
and

(Peptide II)

Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-  
Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-  
Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-  
Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-  
X;

(Peptide V)

- (ii) a negative control sample; and  
(iii) an inactivated HCV positive control sample;  
(iv) specimen diluent;  
(v) enzyme labelled antibodies to human IgG; and  
(vi) an enzyme substrate.

36  
20. An ELISA test kit for the detection of antibodies  
to HCV or NANBHV or the diagnosis of HCV or NANBHV infection  
comprising:

(i) [wherein the] a solid phase coated with a  
peptide composition [comprises] comprising a  
mixture of

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-  
Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-  
Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-  
Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-  
Leu-Gly-Leu-X; and

(Peptide II)

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-  
Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-  
Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-  
Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-  
Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-  
Gln-Pro-Arg-Gly-Arg-Arg-X;

(Peptide VIII)

- (ii) a negative control sample; and

- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.

31 (Twice Amended). An ELISA test kit for the detection of antibodies to HCV or NANBHV or the diagnosis of HCV or NANBHV infection comprising:

- (i) [wherein the] a solid phase coated with a peptide composition [comprises] comprising a mixture of

Ser-Gly-Lys-Pro-Ala-Ile-Ile-Pro-Asp-Arg-Glu-Val-Leu-Tyr-Arg-Glu-Phe-Asp-Glu-Met-Glu-Glu-Cys-Ser-Gln-His-Leu-Pro-Tyr-Ile-Glu-Gln-Gly-Met-Met-Leu-Ala-Glu-Gln-Phe-Lys-Gln-Lys-Ala-Leu-Gly-Leu-X;

(Peptide II)

Lys-Gln-Lys-Ala-Leu-Gly-Leu-Leu-Gln-Thr-Ala-Ser-Arg-Gln-Ala-Glu-Val-Ile-Ala-Pro-Ala-Val-Gln-Thr-Asn-Trp-Gln-Lys-Leu-Glu-Thr-Phe-Trp-Ala-Lys-His-Met-Trp-Asn-Phe-X; and

(Peptide V)

Ser-Thr-Ile-Pro-Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln-Asp-Val-Lys-Phe-Pro-Gly-Gly-Gly-Gln-Ile-Val-Gly-Gly-Val-Tyr-Leu-Leu-Pro-Arg-Arg-Gly-Pro-Arg-Leu-Gly-Val-Arg-Ala-Thr-Arg-Lys-Thr-Ser-Glu-Arg-Ser-Gln-Pro-Arg-Gly-Arg-Arg-X;

(Peptide VIII);

- (ii) a negative control sample; and
- (iii) an inactivated HCV positive control sample;
- (iv) specimen diluent;
- (v) enzyme labelled antibodies to human IgG; and
- (vi) an enzyme substrate.

#### REMARKS

Applicant takes this opportunity to express its appreciation for the Examiner's cooperation and the Examiner's Amendment.

In the present Rule 312 Amendment, Claims 1 and 10 were amended to add an upper limit to the range of relative immunoreactivity to antibodies to HCV of the segments. Similar additions were made pursuant to the Examiner's Amendment of November 21, 1991, amending Claims 1, 11, 37 and 42. Moreover, such amendment finds support in the specification in Table 1 on page 31 where the range of relative immunoreactivities for various segments were described. In particular, Peptide II segments range from 3.1 to 101%, thus providing support for the amendment adding "up to 101%."

The amendment is made to conform the claims to the Examiner's Amendment so that the claimed subject matter is consistent throughout.

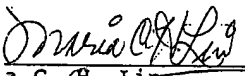
Claims 59, 60 and 61 were amended to add a "solid phase coated with the peptide composition." This amendment finds support in Examples 1, 2, 8, 9, 10, 12, 14, 15 and 18 which disclose the use of peptide compositions to coat a solid phase to perform the ELISA technique. The amendment is made to put the claims 59, 60 and 61 into correct form.

It is respectfully submitted that no new matter or new issues requiring a search have been introduced. Entry of the amendment is hereby requested.

Respectfully submitted,

MORGAN & FINNEGAN

Dated: December 11, 1991

By:   
Maria C. H. Lin  
Reg. No. 29,323

MORGAN & FINNEGAN  
345 Park Avenue  
New York, NY 10154  
(212) 758-4800

1151-4043

PATENT  
U.S.S.N. 07/558,799



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Wang, Chang Yi  
Serial No. : 07/558,799  
Filed : July 26, 1991  
For : SYNTHETIC PEPTIDES SPECIFIC FOR THE DETECTION  
OF ANTIBODIES TO HCV, DIAGNOSIS OF HCV  
INFECTION AND PREVENTION THEREOF AS VACCINES  
Group : 189  
Examiner : Lester Lee

EXPRESS MAIL CERTIFICATE

Express Mail Label No. GB301069659US  
Date of Deposit: December 11, 1991

I hereby certify that the following attached paper(s) or fee:

1. Amendment Pursuant to 37 C.F.R. § 1.312
2. Issue Fee Transmittal
3. Check for \$525.00  
(issue fee of 07/558,799)
4. Post card

are being deposited with the United States Postal Service  
"Express Mail Post Office to Addressee" service under 37 C.F.R.  
§1.10 on the date indicated above and is addressed to the  
Commissioner of Patents and Trademarks, Washington, D.C. 20231.

Francisco Garcia  
(Typed or printed name of  
person mailing paper(s) or fee)

Francisco Garcia  
(Signature of person mailing paper(s) or fee)

Mailing Address:

MORGAN & FINNEGAN  
345 Park Avenue  
New York, New York 10154  
(212) 758-4800



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
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07/558,799 07/26/90 WANG C 1151-4043

MORGAN & FINNEGAN  
345 PARK AVE.  
NEW YORK, NY 10154

EXAMINER  
LEE, L

ART UNIT PAPER NUMBER

1811

14

DATE MAILED:

01/21/92

- A. ☐ The petition filed \_\_\_\_\_ under 37 CFR 1.312(b) is granted.  
The paper has been forwarded to the examiner for consideration on the merits.

- B. ☒ The amendment filed Dec. 11, 1991 under 37 CFR 1.312 has been considered, and has been:

1. ☐ entered
2. ☒ entered as directed to matters of form not affecting the scope of the invention (0.3311).
3. ☐ disapproved. A report appears below.
4. ☐ entered in part. A report appears below.

Report:

*Leslie L. Lee*  
LESLIE L. LEE  
PRIMARY PATENT EXAMINER  
ART UNIT 1811  
1811

PLEASE FURNISH YOUR ZIP CODE IN ALL CORRESPONDENCE



PTO UTILITY GRANT

Paper Number 15

The  
United  
States  
of  
America

The Commissioner of Patents  
and Trademarks

*Has received an application for a patent  
for a new and useful invention. The title  
and description of the invention are en-  
closed. The requirements of law have  
been complied with, and it has been de-  
termined that a patent on the invention  
shall be granted under the law.*

Therefore, this

United States Patent

*Grants to the person or persons having  
title to this patent the right to exclude  
others from making, using or selling the  
invention throughout the United States  
of America for the term of seventeen  
years from the date of this patent, sub-  
ject to the payment of maintenance fees  
as provided by law.*



*Harry F. Marksh, Jr.*

Commissioner of Patents and Trademarks

*Missella A. Keller*

Attest

PTO-1584

Bc





**UNITED STATES DEPARTMENT OF COMMERCE**  
**Patent and Trademark Office**

Address: COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
	02/11/93	WANG	C 1151-4043

EXAMINER	
LEE, L	
ART UNIT	PAPER NUMBER
1811	16

DATE MAILED:

02/18/93

Please find below a communication from the EXAMINER in charge of this application.

Commissioner of Patents.


You are hereby notified under 37 C.F.R. § 1.607(d) that an applicant is seeking to provoke an interference with your Patent No. 5,106,726.

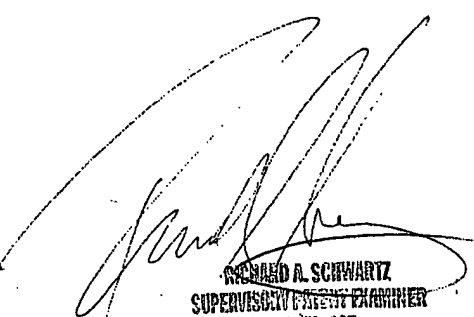
The identity of the applicant will not be disclosed unless an interference is declared.

If a final decision is made not to declare an interference, a notice to that effect will be placed in the patent file and will be sent to the patentee.

If an interference is declared, notice thereof will be made under 37 C.F.R. § 1.611.

Any inquiry concerning this communication from the examiner should be directed to James Ketter whose telephone number is (703) 308-1169.

  
James Ketter  
February 15, 1993

  
RICHARD A. SCHWARTZ  
SUPERVISORY PATENT EXAMINER  
REV 02/18/93



#145

Cap C

1151-4043

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
PATENT EXAMINATION OPERATION

-----X  
In re U.S. Patent No.: 5,106,726 : KBW #16 (YRT)  
Inventor: Chang Yi Wang : Examiner: Lester L. Lee  
Serial No.: 07/558,799 : Group Art Unit: 1811  
Issued: April 21, 1992 :  
For: SYNTHETIC PEPTIDES SPECIFIC :  
FOR THE DETECTION OF :  
ANTIBODIES TO HCV : MAR 17 1993  
-----X

Commissioner of Patents and Trademarks  
Washington, D.C. 20231

REQUEST FOR CERTIFICATE OF CORRECTION PURSUANT TO  
37 C.F.R. 1.323

Dear Sir:

Upon reviewing the above-identified patent, patentee noted certain typographical errors in both the specification and claims which should be corrected. These typographical errors are found in the application as filed by applicants; accordingly the required fee \$100.00 is submitted herewith.

In the Specification:

At Column 19, lines 17 - 19: the first three lines of the amino acid sequence

APPROVED  
AS INDICATED  
AUG 4 1994  
FOR THE COMMISSIONER OF  
PATENTS & TRADEMARKS

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"  
should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --.

4721-1

100 MG 03/11/93 07558799

1 145 100.00 CK

At column 23, lines 46 - 47: the first two lines of the amino acid sequences:

"Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Tyr-  
Trp-Alg-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-  
Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --.

In the Claims:

In Claim 1 (viii), the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --.

In Claim 30, the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --.

It is quite evident after a review of the specification and claims as filed, especially based on the amino acid sequences at columns 21 and 22, Table 7, that the amino acid, sequence

Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr-  
Gly-Asn-Glu-Gly-Cys-Gly-Trp-Ala-Gly-  
Trp-Leu-Leu-Ser-Pro-Arg-Gly-Ser-Arg-  
Pro-Ser-Trp-Gly-Pro-Thr-Asp-Pro-Arg-  
Arg-Arg-Ser-Arg-Asn-Leu-Gly

was intended. (The corrected amino acids are underscored).

Moreover, the entire amino acid sequence of the HCV virus was provided in the specification on Table 6 at Columns 3-4 and 5-6.

The errors now sought to be corrected are inadvertent typographical errors the correction of which does not involve new matter or require examination.

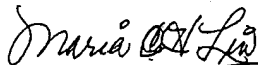
Transmitted herewith is a proposed Certificate of Correction effecting such amendments.

Patentee respectfully solicits the granting of the requested Certificate of Correction.

The Commissioner is hereby authorized to charged any additional fees which may be required for this correction.

Deposit Account No. 13-4500. Order No. 1151-4043. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted



Maria E.H. Lin  
Registration No. 29,323

Date: February 19, 1993

Morgan & Finnegan  
345 Park Avenue  
New York, New York 10154  
Tel. No. (212) 758-4800  
Fax. No. (212) 751-6849

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 5,106,726

Page 1 of 3

DATED : April 21, 1992

INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

At Column 9, lines 17 - 19: the first three lines of the amino acid sequence

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --.

At column 23, lines 46 - 47: the first two lines of the amino acid sequences:

"Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Tyr-  
Trp-Alg-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-  
Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 5,106,726

Page 2 of 3

DATED : April 21, 1992

INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 49, lines 17-19:

In the Claim 1 (viii), the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --

Column 58, lines 18-20:

In the Claim 30, the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 5,106,726

Page 3 of 3

DATED : April 21, 1992

INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

should read: -- Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-  
Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --

This certificate Supersedes Certificate of Correction issued September 20, 1994.

Signed and Sealed this  
Thirteenth Day of December, 1994

Attest:



BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks



UNITED STATES PATENT AND TRADEMARK OFFICE  
CERTIFICATE OF CORRECTION

PATENT NO. : 5,106,726

Page 1 of 3

DATED : April 21, 1992

INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

At Column 4, lines 17 - 19: the first three lines of the amino acid sequence

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- ---.

At column 23, lines 46 - 47: the first two lines of the amino acid sequences:

"Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Tyr-  
Trp-Alg-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-  
Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- ---.

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 5,106,726

Page 2 of 3

DATED : April 21, 1992

INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claim 1 (viii), the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --

In the Claim 30, the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 5,106,726

Page 3 of 3

DATED : April 21, 1992

INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --

Signed and Sealed this  
Twentieth Day of September, 1994

Attest:



BRUCE LEHMAN

Attesting Officer

Commissioner of Patents and Trademarks



UNITED STATES DEPARTMENT OF COMMERCE  
Patent and Trademark Office  
ASSISTANT SECRETARY AND COMMISSIONER  
OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

MAY 3 0 1993

Maria Lin  
MORGAN & FINNEGAN  
345 Park Avenue  
New York, NY 10154

Re: Status Regarding Certificate of Correction for  
U.S. Patent No. 5,106,726

Dear Maria Lin

On February 25, 199, we received a request for a Certificate of Correction for the above-referenced patent. We are currently experiencing a backlog of approximately 6-8 months. We appreciate your patience while we work through our backlog.

Should expedited services be required, please contact me at  
(703) 305-8127.

Sincerely,

Mary Allen, Manager  
Certificates of Correction Branch  
Office of Publication and Dissemination

NOTICE RE: CERTIFICATES OF CORRECTION

Paper No. 17

DATE : 07/14/94

TO : Supervisor, Art Unit 1809 1811

SUBJECT : Certificate of Correction Request in Patent No. 5,106,726

A response to the following question(s) is requested with respect to the accompanying request for a certificate of correction.

- ☐ 1. Would the change(s) requested under 37 CFR 1.323 constitute new matter or require reexamination of the application?
- ☒ 2. Would the change(s) requested under 37 CFR 1.323 materially affect the scope or meaning of the claims allowed by the examiner in the patent?
- ☐ 3. Applicant disagrees with change(s) initialed and dated by Examiner in lieu of an Examiner's Amendment. Should the change request be granted?
- ☒ 4. With respect to the change(s) requested, correcting Office errors, should the patent read as shown in the certificate of correction?
- ☐ 5. If the amendment filed \_\_\_\_\_ had been considered by the Examiner, would the amendment have been entered?

PLEASE RESPOND WITHIN 7 DAYS AND RETURN THE FILE TO ROOM 809, PKI

Y. Townes  
Patent Assistant

TO: CERTIFICATES OF CORRECTION BRANCH

DATE:

The decision regarding the change(s) requested in the certificate of correction is shown below.

- 1. ☐ YES ☐ NO ☐ Comments below
- 2. ☐ YES ☒ NO ☐ Comments below
- 3. ☐ YES ☐ NO ☐ Comments below
- 4. ☒ YES ☐ NO ☐ Comments below
- 5. ☐ YES ☐ NO ☐ Comments below

☒ Comments Note, I was not clear when the claim was needed re column 4, line 17-19

Robert J. Hall  
Supervisor

1811  
Art Unit



UNITED STATES DEPARTMENT OF COMMERCE  
Patent & Trademark Office  
ASSISTANT SECRETARY AND COMMISSIONER  
OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

ATTORNEY ADDRESS

Maria C.H. Lin  
Morgan & Finnegan  
345 Park Avenue  
New York, NY 10154

MAILING DATE AUG 4 1994	
PATENT NO. 5,106,726	PATENT DATE 04/21/92
PATENTEE Chang Yi Wang	
ATTORNEY DOCKET NO. 1151-4043	

NOTIFICATION REGARDING REQUEST FOR CERTIFICATE OF CORRECTION

The Certificate of Correction requested in the patent identified above has been APPROVED with the exception indicated below. The remaining errors will be corrected as requested. The Certificate, so modified, will be issued on SEP 20 1994.

☐ A. THE CHANGES BELOW CANNOT BE INCLUDED IN THE CERTIFICATE SINCE THE REQUEST WAS FILED UNDER RULE 322:

- ☐ 1. Column \_\_\_\_\_, line \_\_\_\_\_, is printed in accordance with the record.
- ☐ (a) The change referred to was initiated and dated by applicant before execution of the application papers.
- ☐ 2. In column \_\_\_\_\_, line \_\_\_\_\_, the error resulted from applicant's failure to comply with Rule 121(a), in that the precise point of entry of the amendment was omitted.
- ☐ 3. In column \_\_\_\_\_, line \_\_\_\_\_, the alleged error is due to applicant's failure to comply with Rule 121(b), wherein provision is made for use of brackets, instead of parentheses, to cancel subject matter and for the use of interlineations to indicate new subject matter.
- ☐ 4. Omission of the priority data from the patent resulted from applicant's failure to fully comply with 35 U.S.C. 119, in that:
- ☐ (a) The priority data was omitted from the oath, or declaration
- ☐ (b) The claim for priority was not included in the application papers.
- ☐ (c) The certified copy of the foreign application was not filed.
- ☐ 5. Since, the inventor name(s) is/are printed in accordance with the type written signature, no correction is in order here, unless a petition is granted (See Petition filing information below).
- ☐ 6. The assignment data is printed in the patent in accordance with PTO-85b, submitted by applicant at time of payment of the base issue fee, no correction is in order here, unless a petition is granted (See Petition filing information below).

Any petition should be directed to the attention of the Assistant Commissioner for Patents, using the following mailing address or FAX number.

By Mail: Commissioner of Patents and Trademarks  
Box DAC  
Washington, D.C. 20231

OR By FAX: (703) 308-6916  
Attn: Office of Petitions

- ☐ 7. In column \_\_\_\_\_, line \_\_\_\_\_, the error arose because Rule 1.52(a) or 1.52(b) was not complied with. Consequently, words of certain pages were obliterated or not legible causing the Office to provide what appeared to be the proper words.

☐ B. THE REQUEST HAS BEEN CHANGED AS SHOWN BELOW TO COMPLY WITH THE RECORD:

- ☐ 1. The error complained of in column \_\_\_\_\_, line \_\_\_\_\_, occurred in column \_\_\_\_\_, line \_\_\_\_\_, where the changes will be made.
- ☐ 2. The change requested in \_\_\_\_\_ has been modified by:

☒

C. THE FOLLOWING CORRECTION(S) CANNOT BE INCLUDED IN THE CERTIFICATE FOR THE REASONS GIVEN BELOW:

☒☐☐☐☐☐☐☐

1. The word sequence \_\_\_\_\_, purported to be in column 4, line 7-19 cannot be found in the printed patent.
2. The alleged error in column \_\_\_\_\_, line \_\_\_\_\_, is an editing change made in accordance with the style of the Invention Patent Manual.
3. In column \_\_\_\_\_, line \_\_\_\_\_, the alleged error is in fact a change made by the examiner and considered to be in accordance with the permissible amendments enumerated in M.P.E.P. 1302.04.
4. In the title, it is the practice to exclude words such as "Improvements in", "New", "A", "Novel", etc., from the printed patent.
5. Comparison of the patent in column \_\_\_\_\_, line \_\_\_\_\_, with the corresponding location in the application file reveals that there is no discrepancy.
6. The numbering of the claims and their dependency in the printed patent is in accordance with the renumbering of dependent claims by the examiner as described in M.P.E.P. 608.01(n).
7. The alleged error in column \_\_\_\_\_, line \_\_\_\_\_, is a change made in an Examiner's Amendment at time of allowance. Since no error is involved and since applicant filed no objection prior to payment of the base issue fee, the requested change will not be included in the Certificate.
8. The error complained of in column \_\_\_\_\_, line \_\_\_\_\_, cannot be corrected since:

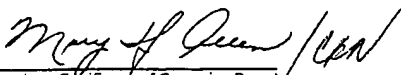
D. ADDITIONAL CORRECTIONS:

E. OTHER (Fee not enclosed):

FOR ADDITIONAL INFORMATION REGARDING THIS NOTIFICATION PLEASE CONTACT:

Your Name  
Certificates of Correction  
(703) 305-

WITHIN 4 WEEKS FROM MAILING DATE OF THIS NOTIFICATION

  
Supervisor, Certificates of Correction Branch

This decision is rendered pursuant to authority delegated by the Solicitor under authority delegated to him by the Commissioner of Patents and Trademarks.

Table  
only 1  
PRINTED MATTER

UNITED STATES PATENT AND TRADEMARK OFFICE  
CERTIFICATE OF CORRECTION

PATENT NO. : 5,106,726

DATED : April 21, 1992

INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification:

At Column 4, lines 17 - 19: the first three lines of the amino acid sequence

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr --.

At column 23, lines 46 47: the first two lines of the amino acid sequences: (Amo A)

"Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Tyr-  
Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-  
Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr --.

1 of 2

MAILING ADDRESS OF SENDER:

Maria C.H. Lin  
Morgan & Finnegan  
345 Park Avenue  
New York, NY 10154

PATENT NO. 5,106,726

No. of add'l. copies  
@ 30¢ per page





Staple  
Here  
Only

PRINTER'S TRIM LINE

UNITED STATES PATENT AND TRADEMARK OFFICE  
CERTIFICATE OF CORRECTION

PATENT NO. : 5,106,726

DATED : April 21, 1992

INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims:

In the Claim 1 (viii), the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-

Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-

Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --

In the Claim 30, the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-

Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-

Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-

Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-

Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- -- 2 of 2

MAILING ADDRESS OF SENDER:

Maria C.H. Lin  
Morgan & Finnegan  
345 Park Avenue  
New York, NY 10154

PATENT NO. 5,106,726

No. of add'l. copies  
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1151-4043

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
PATENT EXAMINATION OPERATION

*2nd request*

In re U.S. Patent No.: 5,106,726 :

Inventor: Chang Yi Wang :

Examiner: Lester L. Lee

Serial No.: 07/558,799 :

Group Art Unit: 1811

Issued: April 21, 1992 :

For: SYNTHETIC PEPTIDES SPECIFIC  
FOR THE DETECTION OF  
ANTIBODIES TO HCV :

RECEIVED  
SEP - 1 94  
CORRECTION  
DEPARTMENT OF  
COMMERCE  
PATENT & TRADEMARK OFF.

Commissioner of Patents  
and Trademarks  
Washington, D.C. 20231

REQUEST FOR CERTIFICATE OF CORRECTION PURSUANT TO  
37 C.F.R. 1.323

Dear Sir:

This is in response to the Notification regarding request  
for Certificate of Correction.

The Examiner has indicated that the sequence purported to be  
in Column 4, line 17-19 cannot be found in the printed patent. A  
review of the Request and the proposed Certificate of Correction  
and the issued patent shows that the location of the error was  
misidentified. The error is to be found in Column 9, lines 17-  
19.

A substitute certificate of correction is enclosed herewith.  
Entry is requested.

The Commissioner is hereby authorized to charged any additional fees which may be required for this correction. Deposit Account No. 13-4500. Order No. 1151-4043. A DUPLICATE COPY OF THIS SHEET IS ATTACHED.

Respectfully submitted

MORGAN & FINNEGAN

Date: August 25, 1994

Morgan & Finnegan  
345 Park Avenue  
New York, New York 10154  
Tel. No. (212) 758-4800  
Fax. No. (212) 751-6849

By: Maria C.H. Lin  
Registration No. 29,323

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 5,106,726  
DATED : April 21, 1992  
INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Specification:

At Column 9, lines 17 - 19: the first three lines of the amino acid sequence

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- ---.

At column 23, lines 46 - 47: the first two lines of the amino acid sequences:

"Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Tyr-  
Trp-Alg-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Ile-Pro-Lys-Val-Arg-Arg-Pro-Glu-Gly-Arg-Thr-  
Trp-Ala-Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- ---.

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 5,106,726  
 DATED : April 21, 1992  
 INVENTOR(S) : Chang Yi Wang

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims:

In the Claim 1 (viii), the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
 Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
 Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
 Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
 Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- --

In the Claim 30, the first three lines of the amino acid sequence:

"Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
 Arg-Arg-Pro-Glu-Gly-Arg-Tyr-Trp-Ala-  
 Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Thr-"

should read: -- Gly-Arg-Arg-Gln-pro-Ile-Pro-Lys-Val-  
 Arg-Arg-Pro-Glu-Gly-Arg-Thr-Trp-Ala-  
 Gln-Pro-Gly-Tyr-Pro-Trp-Pro-Leu-Tyr- -- 2 of 2

MAILING ADDRESS OF SENDER:

Maria C.H. Lin  
 Morgan & Finnegan  
 345 Park Avenue  
 New York, NY 10154

PATENT NO. 5,106,726

No. of add'l copies  
 @ 50¢ per page



1151-4043

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
PATENT EXAMINATION OPERATION

-----X  
In re U.S. Patent No.: 5,106,726 :  
Inventor: Chang Yi Wang : Examiner: Lester L. Lee  
Serial No.: 07/558,799 : Group Art Unit: 1811  
Issued: April 21, 1992 :  
For: SYNTHETIC PEPTIDES SPECIFIC :  
FOR THE DETECTION OF :  
ANTIBODIES TO HCV :  
-----X

Commissioner of Patents and Trademarks  
Washington, D.C. 20231

CERTIFICATE OF MAILING (37 C.F.R. 1.8a)

Dear Sir:

I hereby certify that the attached

1. Certificate of Correction (2 pages);
2. Request for Certificate of Correction Pursuant  
to 37 CFR 1.323;
3. Certificate of Mailing; and
4. Postcard

along with any paper(s) referred to as being attached or  
enclosed) and this Certificate of Mailing are being deposited  
with the United States Postal Service on the date shown below  
with sufficient postage as first-class mail in an envelope  
addressed to the: Commissioner of Patents and Trademarks,  
Washington, D.C. 20231.

Respectfully submitted

MORGAN & FINNEGAN

Date: August 25, 1994

Morgan & Finnegan  
345 Park Avenue  
New York, New York 10154  
Tel. No. (212) 758-4800  
Fax. No. (212) 751-6849

By: *Maria C.H. Lin*  
Maria C.H. Lin  
Registration No. 29,323

FORM PTO-875 REV. 1-85	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	SERIAL NO. <b>558799</b>	FILING DATE <b>7-26-90</b>
PATENT APPLICATION FEE DETERMINATION RECORD		APPLICANT (FIRST NAMED) <b>WANG</b>	

### CLAIMS AS FILED - PART I

OR	NO FILED	NO EXTRA
BASIC FEE		
ADJ. CLAIMS	<b>43</b> 20	<b>23</b>
INDEP. CLAIMS	<b>5</b> 3	<b>2</b>
MULTIPLE DEPENDENT CLAIM PRESENT		

If the difference in col. 1 is less than \$20, enter "0" in col. 2

#### SMALL ENTITY

RATE	FEE
	\$ 185
x 6	\$ 138
x 18	\$ 36
60	\$
TOTAL	\$ <b>374</b>

#### OTHER THAN A SMALL ENTITY

RATE	FEE
	\$ 370
x 12	\$
x 36	\$
120	\$
TOTAL	\$

### CLAIMS AS AMENDED - PART II

(1)	(2)	(3)
CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA
TOTAL <b>40</b>	MINUS <b>43</b>	<b>—</b>
INDEP <b>18</b>	MINUS <b>5</b>	<b>13</b>
FIRST PRESENTATION OF MULTIPLE DEP. CLAIM		

#### SMALL ENTITY

RATE	ADDIT. FEE
x 6	\$
x 18	\$ <b>370</b>
60	\$
TOTAL	\$ <b>370</b>

#### OTHER THAN A SMALL ENTITY

RATE	ADDIT. FEE
x 12	\$
x 36	\$ <b>780</b>
120	\$
TOTAL	\$

(1)	(2)	(3)
CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA
TOTAL <b>37</b>	MINUS <b>40</b>	<b>—</b>
INDEP <b>18</b>	MINUS <b>18</b>	<b>—</b>
FIRST PRESENTATION OF MULTIPLE DEP. CLAIM		

RATE	ADDIT. FEE
x 6	\$
x 18	\$
60	\$
TOTAL	\$

RATE	ADDIT. FEE
x 12	\$
x 36	\$
120	\$
TOTAL	\$

(1)	(2)	(3)
CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NO. PREVIOUSLY PAID FOR	PRESENT EXTRA
TOTAL	MINUS	
INDEP	MINUS	
FIRST PRESENTATION OF MULTIPLE DEP. CLAIM		

RATE	ADDIT. FEE
x 6	\$
x 18	\$
60	\$
TOTAL	\$

RATE	ADDIT. FEE
x 12	\$
x 18	\$
60	\$
TOTAL	\$

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